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Peptides 20 (1999) 1247–1262
Review article

# Luteinizing hormone-releasing hormone analogs: their impact on the control of tumorigenesis☆

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Received 13 May 1998; accepted 21 April 1999

**Abstract**

The development of the luteinizing hormone-releasing hormone (LH-RH) agonists and antagonists and the principles of their clinical use were reviewed. In the 28 years that have elapsed since the elucidation of the structure of LH-RH, various applications in gynecology, reproductive medicine, and oncology have been established for LH-RH agonists and antagonists. These clinical applications are based on inhibition of the pituitary and the gonads. The advantage of the LH-RH antagonists is due to the fact that they inhibit the secretion of gonadotropins and sex steroids immediately after the first injection and thus achieve rapid therapeutic effects in contrast to the agonists, which require repeated administration. LH-RH antagonists should find applications in the treatment of benign gynecologic disorders and benign prostatic hypertrophy and in assisted reproduction programs. The primary treatment of advanced androgen-dependent prostate cancer is presently based on the use of depot preparations of LH-RH agonists, but antagonists like Cetrorelix already have been tried successfully. Antagonists of LH-RH might be more efficacious than agonists in treatment of patients with breast cancer as well as ovarian and endometrial cancer. Recently, practical cytotoxic analogs of LH-RH that can be targeted to LH-RH receptors on tumors have been synthesized and successfully tested in experimental cancer models. Targeted cytotoxic LH-RH analogs show a great promise for therapy of prostate, breast, and ovarian cancers. 

*Keywords:* LH-RH agonists and antagonists; Cytotoxic LH-RH analogs; Endometrial; Ovarian; Breast and prostate cancer

## 1. Introduction

More than 25 years have passed since my laboratory first isolated hypothalamic luteinizing hormone-releasing hormone (LH-RH), identified its structure, and synthesized it [4,109,110,146–148,153,159]. After I announced the structure of porcine LH-RH (Fig. 1) at the Endocrine Society meeting in San Francisco in June 1971 [146], it was synthesized by Guillemin's group [116]. The following year, they reported the amino acid sequence of ovine LH-RH [16], which proved to be the same as that of pig LH-RH. Subsequent studies showed that the structure of hypothalamic LH-RH in all mammalian species examined, including human, is identical. Mammalian LH-RH is also active in birds and in some species of fish, but at least 12 additional molecular forms of LH-RH that differ structurally have been identified in birds, reptiles, amphibians, fishes, other vertebrates, and protochordata [133]. Another isoform of decapeptide LH-RH—LH-RH-II—also has been reported in mammalian brain.

LH-RH is the primary link between the brain and the pituitary in the regulation of gonadal functions and plays a pivotal role in vertebrate reproduction. Because both natural LH-RH and the synthetic decapeptide corresponding to its structure possessed major follicle-stimulating hormone (FSH)-releasing as well as LH-releasing activity, we put forward a concept that one hypothalamic hormone, designated LH-RH/FSH-RH or simply gonadotropin-releasing hormone (Gn-RH) [148], controls the secretion of both gonadotropins from the pituitary gland. Although LH-RH is now accepted as the main FSH-releasing hormone, for reasons of historical continuity the abbreviation LH-RH was recommended for naming its analogs [154,156]. In addition, the abbreviation Gn-RH for gonadotropin-releasing hormone is confusing because it is too similar to GH-RH

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☆ Presented as a plenary lecture at the 10th World Congress on Human Reproduction, May 5, 1999, Salvador, Bahia, Brazil.

0196-9781/99/$ – see front matter 
PII: S0196-9781(99)00130-8

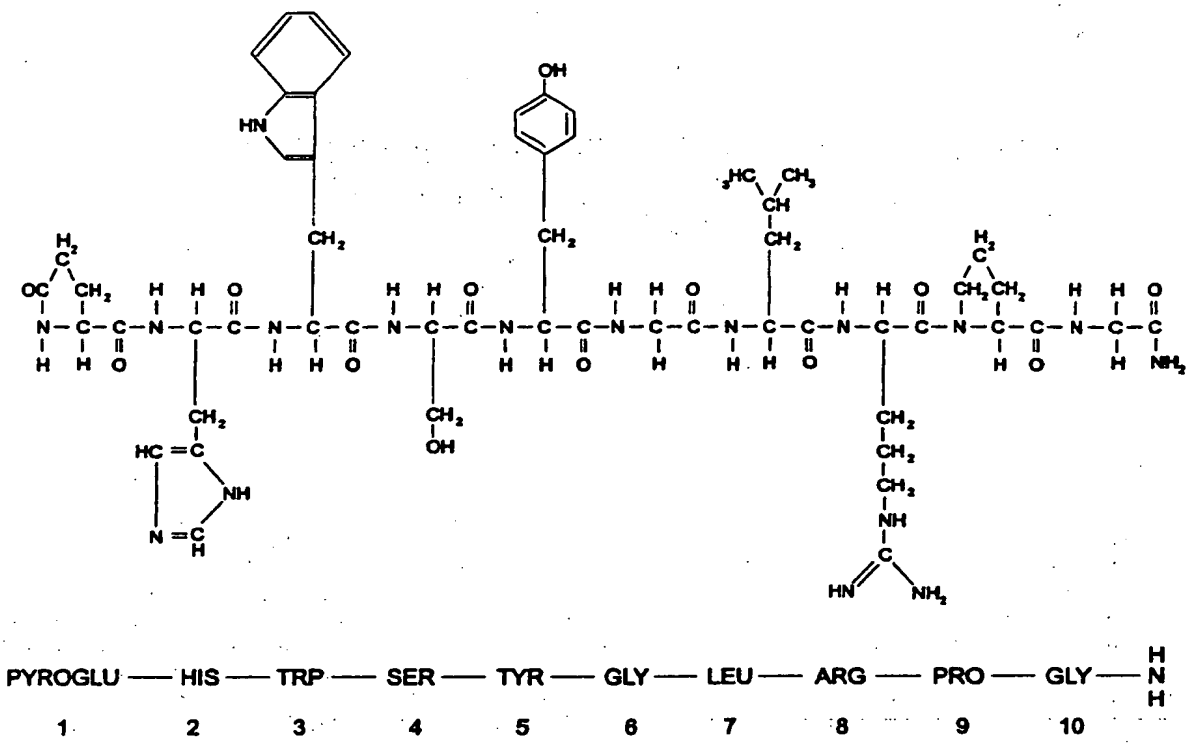


Fig. 1. The molecular structure of LH-RH.

(growth hormone-releasing hormone), for which many agonistic and antagonistic analogs already exist [156].

Even before synthetic LH-RH became available, we clearly demonstrated in clinical studies carried out in 1968 and 1969 in Mexico that highly purified porcine LH-RH, LH, and FSH release in men and women under a variety of conditions [81,82,153]. Subsequently, various clinicians, including our collaborators, carried out extensive clinical studies with synthetic LH-RH [154,155]. LH-RH was utilized diagnostically to determine the pituitary LH and FSH reserve, as well as therapeutically for induction of ovulation in amenorrheic women and treatment of oligospermia and cryptorchidism [155]. At present, synthetic LH-RH is used mostly for evaluating hypothalamic-pituitary gonadotropin function and for induction of ovulation.

### *1.1. Development of analogs of LH-RH*

In 1971, we also postulated that replacement of one or more amino acids in LH-RH might result in analogs with increased LH-releasing activity or antagonistic action [152]. Since 1972, systematic work has been proceeding to synthesize agonistic and antagonistic analogs of LH-RH. A powerful interest in medical applications of LH-RH derivatives stimulated this undertaking. Thus, the intense activity that has occurred in this field was caused by the desire to synthesize superactive analogs with prolonged biologic activity that would be more useful therapeutically than LH-RH itself and to develop antagonistic analogs that were intended at first for contraception and subsequently for gynecological and oncological use [149,155]. However, at that time, we could not imagine the impact and the variety of application, including major uses in oncology, that LH-RH analogs would turn out to have. In the past 25 years, more than 3000 analogs of LH-RH have been synthesized [5,31,32,80,139,144,149–151,177]. Many agonistic analogs more potent than the parent hormone have been made [31, 80,144,149,150,155,177]. Several of these analogs are being used clinically, and the list of their applications is steadily expanding. Potent antagonists of LH-RH such as Cetrorelix [5,139,150,151] that are suitable for clinical use have also been synthesized. In the past few years, diverse cytotoxic analogs of LH-RH have been developed in our laboratory. These analogs consist of cytotoxic radicals, such as doxorubicin, linked to LH-RH agonists that function as carriers that can be targeted to LH-RH receptors on tumors [118]. In experimental studies, these analogs eradicated various tumors and their metastases. LH-RH antagonists and cytotoxic analogs appear to be important additions to clinical armamentarium.

Thus, the discovery of LH-RH has led to many practical clinical uses, and analogs of LH-RH have various important

**D-Trp$^6$-LH-RH**

**pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-$NH_2$**

Fig. 2. The molecular structure of [D-Trp6]LH-RH.

applications in gynecology and oncology. I will now review some selected experimental and clinical findings on the agonistic, antagonistic, and cytotoxic analogs of LH-RH. Various uses of LH-RH analogs in reproductive medicine have been covered recently by others and by me [145], so I will cite those topics only very briefly and will concentrate on applications of the agonists, antagonists, and cytotoxic analogs of LH-RH in cancer therapy.

### *1.2. Agonists of LH-RH*

The half-life of LH-RH is very short—about 2–4 min—and more potent and longer-acting analogs were considered to be necessary for clinical applications. The studies on the relationship between structure and biologic activity showed that histidine in position 2 and tryptophan in position 3 play a functional role in the biologic activity of LH-RH, and simple substitutions or deletions in this active center decrease or abolish LH-RH activity [31,80,155]. However, considerable activity can be obtained by the substitution of these amino acids by structures possessing similar acid-base and hydrogen-bonding capacity, or suitably oriented aromatic nuclei capable of generating similar electronic interactions. Positions 2 and 3 are also the preferred ones for substitution to generate inhibitory activity. However, the tripeptide pyro-Glu-His-Trp or its amide are inactive. Amino acids in positions 1 and 4–10 are essential for binding to the receptors and exerting conformational effects [31,80,155]. Substitutions in positions 6 and 10 can lead to superactive peptides. Thus, several LH-RH analogs substituted in positions 6, 10, or both are much more active than LH-RH and also possess prolonged activity [24,31,46,80,144, 149,150,177]. Of these, the most important are: [D-Trp$^6$]LH-RH (Decapeptyl®, triptorelin) (Fig. 2), [D-Leu$^6$,Pro$^9$-NHET]LH-RH (Leuprolide, Lupron™), [D-Ser(Bu$^t$)$^6$,Pro$^9$-NHET]LH-RH (Buserelin), [D-Ser(Bu$^t$)$^6$,Aza-Gly$^{10}$]LH-RH (Zoladex™, goserelin), and [D-Nal(2)$^6$]LH-RH (Nafarelin), which are 50–100 times more potent than LH-RH [24,31,46, 80,144,149,150,177].

This greater biologic activity of the analogs is due both to increased resistance to enzymatic degradation and an enhancement in receptor affinity. The substitution of Gly6 by D-amino acids renders the analog more resistant to degradation by endopeptidases, which cleave LH-RH at this position and which are widely distributed in mammalian tissues [80].

### *1.3. Principles of oncological and gynecological use of LH-RH agonists*

Although an acute injection of superactive agonists of LHRH induces a marked and sustained release of LH and FSH, paradoxically, chronic administration produces inhibitory effects [22,31,71,80,142,144,149,150,177]. This can be explained by the findings that LH-RH secretion is pulsatile and physiologic stimulation of secretion of gonadotropins requires intermittent LH-RH release [11,89]. Continuous stimulation of the pituitary by chronic administration of LH-RH or its superactive agonists produces inhibition of hypophyseal-gonadal axis through the process of down-regulation (a reduction in the number) of pituitary receptors for LH-RH, decrease in expression of LH-RH receptor gene, desensitization of the pituitary gonadotrophs, and a suppression of circulating levels of LH and sex steroids [21,39,119,144,149–151]. The molecular and cellular basis of the LH-RH action on the pituitary and signal transduction pathways of LH-RH receptors have been reviewed expertly [21,168]. The cloning of cDNA for mouse, rat, and human LH-RH receptor and the organization of LH-RH receptor gene have been reported [77,167].

These processes that can be produced by repeated administration or depot preparations of LH-RH agonists have important clinical applications. Treatment of central precocious puberty, polycystic ovarian disease, hirsutism, and the use for in vitro fertilization and embryo-transfer programs are based on the suppression of gonadotrophin secretion (selective medical hypophysectomy). Therapy of sex hormone-dependent malignant neoplasms, typified by prostate and breast cancer, endometrial carcinoma, as well as of other diseases or conditions such as benign prostate hyperplasia, uterine leiomyomas, and endometriosis, is based on the reversible medical castration and the creation of a state of sex steroid deprivation.

LH-RH analogs also exert direct inhibitory effects on breast, prostate, ovarian, and endometrial cancers mediated through specific LH-RH receptors on the tumor cells [29, 34–36,40,100,134,150,151,167]. These effects are further discussed in the sections on specific neoplasms. The existence of functional regulatory LH-RH loops in prostate cancer and ovarian cancer also has been postulated [29,34]. These systems would consist of locally produced LH-RH-like peptides and specific LH-RH receptors [29,34,35].

### *1.4. Antagonists of LH-RH*

The design of modified structures that might compete with a biologically active compound for the same receptor sites, and yet exhibit little intrinsic activity, is a classic concept that has been used to develop a number of drugs, such as sulfanilamide and 5-fluorouracil. Since 1972, hundreds of LH-RH antagonists have been synthesized and assayed in animals [31,32,80,144,149,150,177]. Early inhibitory analogs were hydrophilic and contained replace-

**[Ac-D-Nal(2)$^1$,D-Phe(4Cl)$^2$,D-Pal(3)$^3$,D-Cit$^6$,D-Ala$^{10}$]LH-RH**

**Acetyl-D-2-naphthylalanyl-D-4-chlorophenylalanyl-D-3-pyridylalanyl-seryl-tyrosyl-D-citrullyl-leucyl-arginyl-prolyl-D-alanine amide**

Fig. 3. The LH-RH antagonist Cetrorelix.

ments or deletions for His in position 2 and Trp in position 3 [80,140,149,155]. Later, it was found that incorporation of a D-amino acid in position 6 increased the inhibitory activity [80,155]. [D-Phe$^2$,D-Trp$^3$,D-Phe$^6$]LH-RH was the first inhibitory analog found to be active in men and women [17,52, 144,149,155]. Insertion of D-arginine in position 6 of LH-RH antagonists increased the inhibitory activity [80, 144,149]. However, hydrophilic antagonists with D-Arg or related basic residues in position 6 induced histamine liberation resulting in transient edema and other anaphylactoid reactions [32,80,144,149].

To eliminate the undesirable edematogenic effect, new analogs with neutral D-ureidoalkyl amino acids, such as D-Cit at position 6, were synthesized in our laboratory [5]. Among these antagonists devoid of any significant edematogenic effects, Nal(2)$^1$,D-Phe(4Cl)$^2$,D-Pal(3)$^3$,D-Cit$^6$,D-Ala$^{10}$]LH-RH (SB-75) (Cetrorelix; Fig. 3) had the highest overall inhibitory activity and receptor binding affinity [5,139,144,150].

Other groups have also reported different structural modifications that preserve high activity and diminish anaphylactoid activity. Antagonists like antide Phe(4Cl)$^2$,D-Pal(3)$^3$,Lys(Nic)$^5$,D-Lys(Nic)$^6$,Lys(iPr)$^8$,D-Ala$^{10}$]LH-RH [103] and Nal-Glu antagonist [Ac-D-Nal(2)$^1$, D-Phe(4Cl)$^2$,D-Pal(3)$^3$,Arg$^5$,D-Glu$^6$(AA),D-Ala$^{10}$]LH-RH [141] are potent, although antide has low solubility and Nal-Glu antagonist caused some clinical side effects. Among other antagonists that are being developed are Azaline B [Ac-D-Nal$^1$,D-Phe(4Cl)$^2$,D-Pal$^3$,Aph$^5$(Atz),Aph$^6$(Atz),ILys$^8$,D-Ala$^{10}$]-GnRH [70], Ganirelix [*N*-Ac-D-Nal(2)$^1$,D-*p*-Cl-Phe$^2$,D-Pal(3)$^3$,D-hArg(Et$_2$)$^6$,L-hArg(Et$_2$)$^8$,D-Ala$^{10}$]-LH-RH [120], and Abarelix (PPI-149) [*N*-Ac-D-Nal(2)-D-(*p*-Cl)-Phe-D-Pal(3)-Ser-NM-Tyr-Asn-Leu-ILys-Pro-Gly-NH$_2$ [115].

In Phase I clinical studies, Cetrorelix given i.v., subcutaneously (s.c.), or intramuscularly (i.m.) in doses of 300–1200 μg produced a prompt inhibition of LH and FSH release in postmenopausal women and caused no side effects [55]. Normal men showed a major fall in serum LH, FSH, and testosterone levels for 12–24 h after s.c. administration of 300 μg of Cetrorelix [55]. Behre et al. [8] and Klingmuller et al. [88] obtained similar effects with Cetrorelix in normal men. Cetrorelix appears to have the higher suppressive rate than other LH-RH antagonists and even in large doses of up to 10 mg only occasionally causes minimal erythema [8,88].

LH-RH antagonists should have major uses in gynecology and oncology. LH-RH antagonists produce a competitive blockade of LH-RH receptors, preventing a stimulation by endogenous LH-RH, and cause an immediate inhibition of the release of gonadotropins and sex steroids [80,139, 144,149–151] in contrast to the LH-RH agonists that require repeated administration to achieve this effect. The advantage of the antagonists is based on the fact that they induce inhibition of LH, FSH, and sex steroid secretion from the start of the administration and greatly reduce the time of the onset of therapeutic effects. The use of antagonists prevents a clinical flare-up of disease caused by a transient LH and sex steroid release, which can occur in some cancer patients during initial agonist administration, even when microcapsules are used [80,138,144,149,150]. The principal mechanism of action of LH-RH antagonists was thought to be based on a competitive receptor occupancy of LH-RH receptors, but recently, we have demonstrated that chronic administration of LH-RH antagonist Cetrorelix to rats also produces desensitization of gonadotropes, down-regulation of pituitary LH-RH receptors, and a decrease in the levels of mRNA for LH-RH receptors [130]. Thus some mechanisms of down-regulation of pituitary LH-RH receptors produced by antagonists of LH-RH appear to be similar to those resulting from the agonists [130]. This view is supported by some clinical findings.

### *1.5. Sustained delivery systems for LH-RH analogs*

Initially, agonists of LH-RH were administered daily by the s.c. route or intranasally [150]. However, daily administration is inconvenient. Subsequently, long-acting delivery systems for [D-Trp6]LH-RH (Decapeptyl) and other agonists in microcapsules of poly(DL-lactide-co-glycolide) or different polymers were developed [31,138,144,149,150]. These microcapsules were designed to release a controlled dose of the peptide (usually 100 μg) over a 30-day period [138]. These spherical microcapsules contain 2–6% analog and 94–98% of biodegradable, biocompatible polymer. Other forms of sustained delivery system consist of microgranules (microparticles) of amorphous shape or cylindrical rods containing the peptide analogs.

For administration, the microcapsules or microgranules are suspended in an injection vehicle containing 2% carboxymethylcellulose or D-Mannitol and 1% Tween 20 or 80 in water and injected once per month i.m. through a 20-gauge needle. Preparations of Lupron depot microspheres containing 3.75–7.5 mg of Leuprorelin acetate injectable i.m., or of Zoladex (Goserelin, 3.6 mg) in cylindrical rods of

the polymer poly(DL-lactide-co-glycolide) [31] injectable s.c. through a 16-gauge needle, and polyhydroxybutyrate tablets containing 3.6–5 mg of Buserelin, which is implantable s.c., are also available [31,150]. Improved depot preparations, which release the analogs for 60–90 days, have been developed recently. Microcapsules and other sustained delivery systems permit the delivery of peptides into the blood stream at a controlled rate over an extended period of time. The delivery systems developed for administration of LH-RH analogs are practical and convenient and ensure patient compliance [150]. Sustained delivery systems for LH-RH antagonists Cetrorelix, Abarelix, and other antagonists also are being developed.

### *1.6. Targeted cytotoxic analogs of LH-RH*

An additional new class of antitumor compounds based on LH-RH has been developed consisting of LH-RH analogs, mostly agonists, conjugated to a variety of chemotherapeutic agents [118,157]. It is well known that in prostatic, breast, ovarian, and endometrial cancers conventional chemotherapy is associated with a high toxicity and a varying degree of response. Targeted chemotherapy may be more effective and would greatly reduce the peripheral toxicity of cytotoxic agents. The idea of a 'magic bullet' that could selectively eradicate tumors was originally conceived more than 100 years ago by Paul Ehrlich but remained unexplored for many decades [157]. On the basis of the presence of LH-RH receptors in breast, endometrial, ovarian, and prostatic cancers, we started some 10 years ago the development of targeted antitumor compounds by linking cytotoxic compounds to LH-RH analogs [6,157,158].

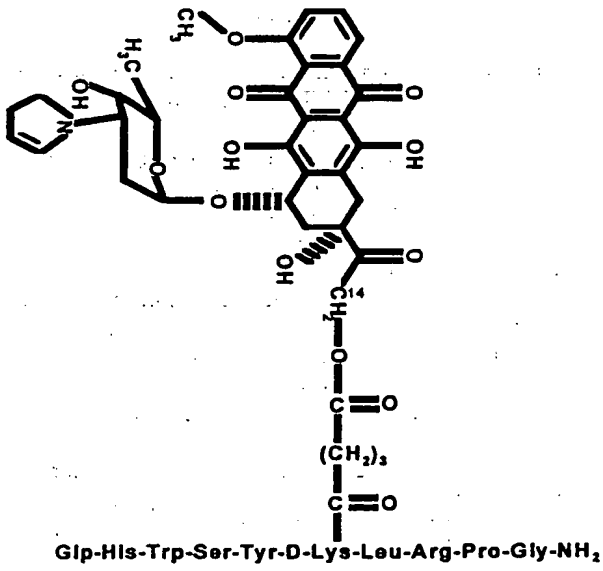


Fig. 4. Molecular structure of cytotoxic LH-RH analog AN-207. [D-Lys$^6$]LH-RH is linked through the ε-amino group of its D-Lys moiety and a glutaric acid spacer to the 14-OH group of 2-pyrrolino-DOX (AN-201). Proc. Natl. Acad. Sci. USA 1996;93;7269–7273. 211 1996, Copyright 1996 National Academy of Sciences. Reprinted with permission of Proceedings of the National Academy of Sciences USA.

Early cytotoxic analogs consisting of various antineoplastic radicals such as D-melphalan, cisplatinum, and methotrexate linked to LH-RH analogs showed binding to prostatic, mammary, endometrial, and ovarian cancer cell lines and inhibited tumor growth in vitro and/or in vivo [6,158]. In some of these early conjugates, doxorubicin (DOX), the most widely used anti-cancer agent, was linked to LH-RH analogs but, unfortunately, the activity of DOX within these hybrids was greatly reduced because of the nature of the linkage [6,158].

Recently, we conjugated [D-Lys$^6$]LH-RH through the ε-amino group of its D-Lys moiety and a glutaric acid spacer to the 14-OH group of DOX to form cytotoxic LH-RH analog AN-152 [118]. An even more potent cytotoxic analog (AN-207) (Fig. 4) was synthesized by linking 2-pyrrolino-DOX (AN-201), a daunosamine-modified derivative of DOX, which is 500–1000 times more active in vitro than its parent compound, to the same [D-Lys$^6$]LH-RH carrier [118]. The antiproliferative activity of the cytotoxic radicals and the high binding affinity of the carrier to LH-RH receptors are both fully preserved in the cytotoxic LH-RH analogs AN-152 and AN-207 [118,157]. We have shown that these new cytotoxic LH-RH analogs AN-152 and AN-207 powerfully inhibit growth of various experimental tumors. This approach, which remains to be tested clinically, could open up a new area of cancer therapy because the cytotoxic analogs developed might have the potential to produce an eventual cure.

### *1.7. Gynecological applications of LH-RH analogs*

LH-RH agonists have been used for more than 12 years in the assisted reproduction (in vitro fertilization and embryo transfer) programs to suppress pituitary-ovarian function [132,179,184]. Recently, Cetrorelix and other antagonists were applied successfully in women undergoing controlled ovarian stimulation procedures [2,28,30,69,99, 122,123,140,175]. LH-RH antagonists appear to have advantages over the agonists for controlled ovarian stimulation-assisted reproduction technology programs.

The lowering of estrogen levels by administration of LH-RH agonists has been used for the management of endometriosis [48,65,111,185] and for treatment of large ovarian endometriomas [23]. Only one incidence of flare-up of disease has been reported [58].

LH-RH agonists have been employed for more than 10 years for the treatment of infertility due to polycystic ovarian disease [3,18,43]. Recently, Hayes et al. showed that administration of an LH-RH antagonist can rapidly suppress LH, FSH, and testosterone levels in women with polycystic ovarian disease [62].

The induction of a state of hypoestrogenism by LH-RH

agonists has been applied for a successful therapy of women with uterine leiomyomas (fibroids) [44,49,63,104,128,176]. Recent results indicate that LH-RH antagonists also can be used for an efficacious medical management of uterine leiomyomas [42,50,85]. Administration of Cetrorelix produces a rapid regression of leiomyomas, and a hysterectomy can be avoided [42,50].

### *1.8. Other nononcological applications of LH-RH analogs*

Benign prostatic hypertrophy (BPH) affects many elderly men and may lead to urinary incontinence. For those patients who are poor operative risks, a nonsurgical treatment of BPH would be beneficial. When LH-RH agonists were tried for therapy of BPH [47,129], a reduction in serum testosterone and a decrease in prostate size were obtained, but after the cessation of the treatment a regrowth of the prostate occurred [129]. Recent clinical trials indicate that LH-RH antagonists like Cetrorelix can produce a long-term improvement in patients with symptomatic BPH [19, 56]. Thus, in addition to lowering the levels of serum testosterone, Cetrorelix appears to exert some inhibitory effect on growth factors, as suggested by our experimental work [96,150].

Various attempts have been made to develop methods for female contraception based on the use of LH-RH agonists and antagonists [13,57,121,144,149,183], but clinical regimens are lacking. It is also doubtful that LH-RH agonists will be suitable as male contraceptives [10,14,64,102]. The combined use of LH-RH antagonists and androgens for the suppression of spermatogenesis in men has been demonstrated [7–9,174], but the acceptability of these methods for male contraception is questionable.

The efficacy of agonists of LH-RH for the treatment of children with precocious puberty is well established [20,27, 61,79,84,94,106,168]. The cessation of sexual development and other clinical benefits to children are clearly documented [20,21,27,61,79,84,94,106,168]. LH-RH antagonists have not been investigated in children with precocious puberty but could achieve a more rapid inhibition of pituitary-gonadal axis.

## 2. Analogs of LH-RH in oncology: experimental studies and clinical applications

### *2.1. Endometrial cancer*

Endometrial cancer is the second most common gynecologic cancer in the western world, ranking only behind breast cancer [39]. Surgery or radiotherapy is successful in 75% of cases, but new methods are needed for advanced (FIGO Stage III or IV) or relapsed cancers [39,150]. Endometrial carcinoma is estrogen-dependent, and thus it should respond to therapy with LH-RH analogs [150]. In addition, high affinity receptors for LH-RH are present on nearly 80% of membranes of human endometrial cancers and human HEC-1A and Ishikawa endometrial cancer cell lines [39,40, 166]. Agonist [D-Trp6]LH-RH and antagonist Cetrorelix inhibited the proliferation of HEC-1A and Ishikawa human endometrial cell lines in vitro [40]. Bioactive and immunoreactive LH-RH and the expression of mRNA for LH-RH were found in these cells [68]. The growth inhibition produced by Cetrorelix was associated with an induction of apoptosis. Cetrorelix also inhibited growth of Ishikawa endometrial tumor cell line in vitro induced by insulin-like growth factor (IGF)-I and -II and inhibited IGF II release from cells [86]. Thus, direct inhibitory effects of LH-RH analogs on the tumor could be taken into consideration in planning the therapy.

Only limited clinical studies have been carried out so far, but in a small Phase II trial in patients with recurrent endometrial cancer, administration of depot preparation of Leuprorelin or Zoladex produced a partial or complete remission in 35% of patients [48]. Phase II trials with microcapsules of [D-Trp$^6$]LH-RH are in progress [39]. These findings provide a strong rationale for the continued exploration of therapeutic approaches based on LH-RH agonists and antagonists such as Cetrorelix in patients with advanced endometrial cancer.

In view of the presence of LH-RH receptors on endometrial cancers, targeted cytotoxic analogs also are being investigated. Preliminary results indicate that cytotoxic analog AN-207 inhibits growth of HEC-1A endometrial cancers xenografted into nude mice [157].

### *2.2. Epithelial ovarian cancer*

Epithelial ovarian cancer is the fourth most frequent cause of cancer-related deaths in women [150]. Treatment based on surgery or chemotherapy is not very effective [150], and mortality rates are increasing. Ovarian cancer may be dependent in part on LH and FSH. In experimental cancer models, the suppression of the secretion of gonadotropins produced by LH-RH agonists inhibited the growth of ovarian tumors [39,144,149,150]. In addition, specific receptors for LH-RH have been found in 78% of surgically removed human ovarian carcinomas [38,39,165] and in EFO-21, EFO-27, and OV-1063 human ovarian cancer cell lines [35,181]. These receptors may mediate direct inhibitory effects of LH-RH analogs on the growth of ovarian cell lines in vitro and in vivo [39,150]; the agonist [D-Trp$^6$]LH-RH at $10^{-9}$ M concentrations significantly reduced proliferation of EFO cell lines in culture [35]. The expression of mRNA for LH-RH and LH-RH receptors in these cell lines [67] supports the view that local LH-RH-like substances may be involved in the proliferation of ovarian cancer [36].

In clinical studies, Parmar et al. have treated patients with advanced ovarian carcinoma (FIGO Stage III and IV) who had relapsed following chemotherapy with microcapsules of [D-Trp$^6$]LH-RH [125,126]. Some patients showed stabilization or partial remission. Bruckner et al. [15] and

others also reported therapeutic responses with Leuprorelin in patients with advanced ovarian cancer. However, in a recent multicenter trial involving approximately 200 patients, no beneficial effects of therapy with microcapsules of [D-Trp$^6$]LH-RH could be found in patients with advanced epithelial ovarian cancer who received surgical cytoreduction and chemotherapy [37].

Various experimental results indicate that Cetrorelix inhibits growth of human OV-1063 epithelial ovarian cancers in nude mice better than agonist [D-Trp$^6$]LH-RH and, therefore, may be more efficacious clinically [35,181]. Studies in vitro indicated that Cetrorelix binds to LH-RH receptors on OV-1063 and EFO-21 cells and can inhibit their proliferation [181]. In nude mice bearing xenografts of OV-1063 and other ovarian cancers, treatment with Cetrorelix inhibited tumor growth and reduced the number of epidermal growth factor (EGF) receptors on tumors and the levels of mRNA for EGF receptors [163,181]. Effects of Cetrorelix on EGF receptors might be related to inhibition of tumor growth [163,181]. Our findings suggest that LH-RH antagonists such as Cetrorelix should be evaluated clinically in patients with epithelial ovarian cancer. Various clinical trials with preparations of Cetrorelix are in progress.

In view of the presence of receptors for LH-RH on ovarian cancers, we evaluated the effects of targeted cytotoxic LH-RH analogs on growth of ovarian cancers [113, 114]. In an initial study, we showed that cytotoxic analog AN-152 inhibited significantly the growth of LH-RH receptor-positive OV-1063 ovarian tumors in nude mice at doses of 20.6 μmol/kg. Equivalent doses of doxorubicin caused substantial mortality. AN-152 did not inhibit the growth of LH-RH receptor-negative UCI-107 human ovarian carcinoma in nude mice [113]. This indicates that the presence of receptors is essential for the targeted cytotoxic therapy. In another study, we investigated the effect of cytotoxic LH-RH analog AN-207 [114]. We demonstrated that the growth of OV-1063 ovarian cancers could be suppressed by administration of 150–250 nmol/kg doses of AN-207 [114]. Cytotoxic radical AN-201 and its mixture with carrier were toxic at these doses and killed all animals. This indicates that targeted cytotoxic LH-RH analog AN-207 is less toxic than equimolar doses of its radical AN-201 and that it can effectively inhibit ovarian tumor growth. These two studies [113,114] suggest that targeted chemotherapy based on analogs such as AN-152 and AN-207 may improve the management of ovarian cancer.

### 2.3. Breast cancer

Breast cancer is the most common malignancy in women, accounting for about one-third of all female cancers. More than 500 000 new cases of breast cancer are reported worldwide each year, and the annual mortality due to this malignancy is about 45 000 in the United States alone [150]. The development of new treatment modalities is necessary.

About 30% of women with breast cancers have estrogen-dependent tumors and can be treated by hormonal manipulations such as Tamoxifen or oophorectomy [39,143,150]. Experimental and clinical studies revealed that agonists of LH-RH might be useful for treatment of estrogen-dependent breast cancer [144,149]. In rat and mouse models of breast adenocarcinoma, chronic administration of agonist [D-Trp$^6$]LH-RH decreased tumor weight and volume [136], suggesting that agonists of LH-RH should be considered for a new hormonal therapy for breast cancer in women [149, 150]. In clinical trials carried out since the early 1980s, regression of tumor mass and disappearance of metastases in premenopausal and some postmenopausal women with breast cancer treated with [D-Trp$^6$]LH-RH, Buserelin, Zoladex, or Leuprorelin have been demonstrated [83,87,105, 107,131,143,178,180].

In clinical trials in France, Mathé et al. treated 23 patients with advanced breast carcinoma with microcapsules of [D-Trp$^6$]LH-RH [107]. All patients showed a decrease in levels of LH. Five of eight pre-menopausal patients were estrogen receptor (ER)-positive, and three of them responded. Three of 15 postmenopausal patients responded; two of them were ER-positive. The results recorded in postmenopausal patients suggest that agonists may have some direct antitumoral action [107].

Williams et al. [180] used Zoladex to treat 53 pre-menopausal patients with advanced breast cancer. Tumor remissions after Zoladex therapy were observed in 31% of patients, the responses occurring primarily in women with well differentiated and ER-positive tumors [180]. In a large trial in 134 premenopausal women with breast cancer, Kaufman et al. [83] utilized depot implant of Zoladex and demonstrated 53% objective tumor responses. Santen et al. [143] summarized various studies with LH-RH agonists and computed a 41% objective response rate in unselective premenopausal patients and 51% in women with ER-positive tumors.

These studies indicate that LH-RH agonists are efficacious for the treatment of premenopausal women with estrogen-dependent, ER-positive breast cancer [149,150]. Combinations of LH-RH agonist with tamoxifen or with chemotherapy also are being investigated in premenopausal women with advanced metastatic breast cancer. The main effect of LH-RH agonists on mammary carcinomas is based on estrogen deprivation, but the response in ≈5–10% of postmenopausal women suggests that some direct antitumor effects of LH-RH analogs are also possible.

Several groups found LH-RH receptors on human breast cancer specimens and in breast cancer lines [33,39,41,112, 150]. Approximately 52% of a large series of breast cancer biopsy samples ($n$ = 500) were found to be positive for LH-RH receptors [41]. Using the human LH-RH receptor c-DNA, Kakar et al. [78] were able to demonstrate the expression of the LH-RH receptor gene in the MCF-7 human breast cancer cell line. It also is well established that LH-RH agonists and antagonists can exert direct antiprolif-

erative effects on human breast cancer lines in vitro [33,39, 150,162].

LH-RH agonists also have been used for the treatment of fibrocystic disease of the breast in premenopausal women [117]. When 66 patients with fibrocystic mastopathy were treated with microcapsules of [D-Trp$^6$]LH-RH for 3–6 months, a response was observed in nearly half of the patients [117].

LH-RH antagonists have been so far tested only in experimental models of breast cancer [136,150,169,182]. In mice bearing MXT estrogen-dependent or -independent mammary adenocarcinomas, Cetrorelix reduced tumor volume and tumor weight and produced regressive changes in the treated tumors that were characteristic of apoptosis (programmed cell death) [169]. In rats bearing 7,12-dimethylbenz[*a*]anthracene-induced mammary carcinomas, Cetrorelix also induced tumor regression [139]. Tumor growth in nude mice bearing transplanted MCF-7 MIII human breast cancers was likewise inhibited by Cetrorelix [182]. This effect was linked to down-regulation of EGF receptors. In collaborative studies, we showed that LH-RH antagonist Cetrorelix can inhibit the growth of MCF-7 breast tumors by interfering with the autocrine action of IGF-II and by directly inhibiting the growth-stimulatory effect of IGFs [66,160]. These experimental findings indicate that Cetrorelix might be effective clinically for the treatment of breast cancer. Clinical trials with Cetrorelix in women with breast cancer are in progress.

Cytotoxic analogs of LH-RH bind with high affinity to human breast cancers [60]. In an initial study on breast cancer, we tested tumor inhibitory action of targeted cytotoxic LH-RH analogs AN-152 and AN-207 in mice bearing estrogen-independent MXT mouse mammary cancers [172]. Analog AN-207 and analog AN-152 given intraperitoneally (i.p.) produced ≈90% inhibition of tumor growth, but equimolar amounts of the cytotoxic radicals were toxic [172]. The advantage of AN-207 is that it is effective in doses ≈150–200 times smaller than AN-152. Recent regimens of treatment for new cytotoxic analogs are based on only one or two injections [76]. Thus, in another investigation, one injection of AN-207 caused a complete regression of MX-1 hormone-independent doxorubicin-resistant human breast cancers in nude mice, which remained tumor-free for at least 60 days after treatment [76]. These results suggest that targeted cytotoxic LH-RH analogs such as AN-207 could be considered for treatment of LH-RH receptor-positive advanced or metastatic breast cancers in women [76].

### *2.4. Prostate cancer*

The greatest therapeutic impact of LH-RH analogs occurred in the field of prostate cancer. Carcinoma of the prostate represents the most common malignancy in the American male and is the second leading cause of cancer-related deaths among adult men [25,127,149,150,161]. Approximately 70% of human prostate cancers are testosterone-dependent [25,127,150,161]. The treatment of advanced (stage C or D) prostate cancer is usually based on androgen dependence of the tumor and includes orchiectomy and administration of estrogens or antiandrogens [25, 127,150,161]. However, surgical castration is associated with a psychological impact, whereas estrogens have cardiovascular, hepatic, and mammotropic side effects and anti-androgens are also toxic to the liver. Approximately 18 years ago, we introduced a new endocrine therapy for advanced prostate cancer based on the use of agonistic analogs of LH-RH [135,173]. Medical castration produced by chronic administration of LH-RH analogs accounts for most benefits derived from this treatment [25,127,149,150,161, 173], but there is also evidence that LH-RH agonists and antagonists can exert direct effects on prostate tumor cells [29,36,100,134,150].

In our initial studies in 1981, we showed that chronic administration of [D-Trp$^6$]LH-RH suppressed tumor growth in rats with Dunning R-327-H prostate cancers and reduced serum levels of LH, FSH, and testosterone [135]. This demonstration led to clinical trials. The efficacy of palliation with agonistic analog of LH-RH in men with advanced prostate cancer was first demonstrated in collaboration with Tolis et al. [173] in a clinical trial that took place in Montreal in 1980–1981. The study of Tolis et al. documented a fall in testosterone levels and marked subjective and objective improvement in patients with stage C or D prostate carcinoma after treatment with agonistic analogs Decapeptyl and Buserelin [173]. These findings have been confirmed and extended by other clinical trials with LH-RH agonists in patients with prostate cancer in Europe and North America [1,25,108,124,127,149,150,161,164]. The LH-RH analogs used clinically for therapy of advanced prostate cancer include Decapeptyl, Buserelin, Leuprolide, and Zoladex [1,25,108,124,127,149,150,161,164]. Initially, agonists of LH-RH were given daily by the s.c. or intranasal route [127,150,161,173]. Subsequently, we developed a long-acting delivery system for [D-Trp$^6$]LH-RH in microcapsules of poly(D-lactide-co-glycolide) designed to release a controlled dose of the peptide over a 30-day period [138]. The efficacy of the slow-release formulation of [D-Trp$^6$]LH-RH microcapsules and other agonists in the treatment of advanced prostatic carcinoma was demonstrated in clinical trials [1,124,150,161]. In a trial in men with prostate cancer carried out with Parmar, Lightman et al., we obtained a 87% objective response in the microcapsule-treated group versus 81% for total orchidectomy [124]. Development of microcapsules and other sustained-release formulations, such as implants that can be administered once a month, made the treatment of patients with prostate cancer more convenient, practical, and efficacious [1,124,150,161]. These regimens also ensure better patient compliance than daily injections. Side effects caused by chronic administration of LH-RH agonists include impotence, loss of libido, and hot flushes and are due to androgen deficiency. Occasional flare-up in

the disease with an increase in bone pain during the first week of administration of agonists has been reported in ≈10% of patients [92,161,164]. This flare-up can be prevented by antiandrogens. Acceptance of LH-RH analogs is excellent. The therapy with agonists of LH-RH is presently the preferred method of treatment for men with advanced prostate cancer, and recent surveys indicate that in ≈70% of cases, LH-RH agonists are selected for primary treatment [25,150].

Labrie [93] introduced the concept of total androgen blockade and proposed the use of a combination of LH-RH agonist with antiandrogen for the treatment of prostate cancer. Combinations of LH-RH agonists with antiandrogens such as flutamide are being used clinically [25,26,92,93]. The benefits of this combination are still controversial, but there may be a small increase in progression-free survival and median length of survival in patients treated with analogs plus flutamide as compared with those the received analogs and placebo [12,93]. However, the combination of LH-RH agonists and antiandrogen cannot prevent an eventual relapse.

### 2.5. LH-RH antagonists in prostate cancer

The use of LH-RH antagonists would avoid the temporary clinical 'flare-up' of the disease that can occur in ≈10–20% of prostate cancer patients, when the LH-RH agonists are given as single agents [150]. We first investigated inhibitory effects of the antagonist Cetrorelix on the growth of experimental prostate cancers [91]. In rats bearing Dunning R-3327-H prostate carcinoma, Cetrorelix caused a greater inhibition of prostate cancer growth than [D-Trp$^6$]LH-RH [91]. To extend our findings, we treated male nude mice bearing xenografts of human androgen-dependent prostate adenocarcinoma PC-82 with microcapsules of the agonist [D-Trp$^6$]LH-RH or microgranules of Cetrorelix. In mice that received Cetrorelix, there was a greater decrease in tumor weight and volume than that produced by the agonist [137]. Animals treated with Cetrorelix also showed more enhanced apoptosis in prostate tumors and lower serum levels of testosterone and prostate-specific antigen (PSA) than mice given the LH-RH agonist [137]. These studies [91,137] demonstrated the efficacy of Cetrorelix in inhibiting growth of androgen-dependent prostate cancers. We then showed that high doses of Cetrorelix inhibited the growth of androgen-independent human DU-145 and PC-3 prostate cancers transplanted into nude mice, possibly through down-regulation of receptors for EGF [72, 73].

Similarly, in our hands, Cetrorelix could decrease the proliferation of DU-145 and PC-3 tumors in vitro only at very high ($10^{-5}$ M) doses [72,73]. An autocrine regulatory loop consisting of receptors for LH-RH and an LH-RH-like peptide was postulated to exist in DU-145 prostate cancers [29]. Dondi et al. [29] and Limonta et al. [101] reported the inhibition of proliferation of DU-145 cells in vitro by Cetrorelix and agonists Buserelin and Zoladex, but Qayum et al. [134] showed that Buserelin stimulated LNCaP cells but had no effect of DU-145 line. Although direct effects of LH-RH analogs on prostate cancer cells appear to be well documented in vitro [29,100,134], the findings may not be applicable to a clinical setting. Administration of high doses of Cetrorelix can also decrease the levels and mRNA expression of EGF receptors and of IGF-II in PC-3 and DU-145 prostate cancers xenografted into nude mice [72,73,95, 96]. Similar effects of Cetrorelix on IGF-I and -II and on receptors for EGF also occur on other tumors [66,86,160, 163,181,182] and could be important clinically. Similarly, a down-regulation in pituitary receptors for LH-RH produced by Cetrorelix in rats also may occur clinically during long term administration of Cetrorelix in men [9,130].

Clinical trials demonstrated that an inhibition of testosterone and PSA levels and a decrease in prostate size as measured by ultrasonography is achieved in patients with advanced prostatic cancer treated with antagonist Cetrorelix [53,54,56]. In the first study, the response to 500 μg of Cetrorelix given twice per day (b.i.d.) s.c. was evaluated in prostatic cancer patients, stage C or D [56]. Therapy with Cetrorelix produced a decrease in bone pain, relief in urinary outflow obstruction, reversal of the signs of prostatism, reduction in serum testosterone to castration values, and decrease in elevated PSA levels. The second study involved 36 patients with stage D prostate cancer with elevated levels of PSA and bone pain [53]. Group I consisted of 16 patients, who received 500 μg of Cetrorelix b.i.d. s.c. for as many as 37 months. Thirteen patients showed a clinical remission, but later five patients relapsed [53]. Group II included 20 patients who received a loading dose of Cetrorelix, 5 mg b.i.d. s.c. for the first 2 days and thereafter 800 μg b.i.d. s.c. for up to 20 months. Nineteen patients showed a clinical remission, but later three relapsed. Five of six patients who were paraplegic due to metastatic invasion of spinal cord showed neurologic improvement during therapy with Cetrorelix [54]. Cetrorelix may be indicated for patients with prostate cancer and metastases to the spinal cord, bone marrow, and other sites in whom the LH-RH agonists cannot be used as single drugs because of the possibility of flare-up. Clinical improvement and absence of side effects suggest that the LH-RH antagonist Cetrorelix is suitable for the therapy of advanced prostate carcinoma, and BPH and extensive clinical trials are in progress.

In conclusion, it has been documented in thousands of patients with advanced prostate cancer that LH-RH agonists provide an effective palliative therapy resulting in objective stable disease or partial remission. However, all hormonal therapies aimed at androgen deprivation, including castration and LH-RH agonists or antagonists, can provide only a remission with a limited duration, and most patients with advanced prostatic carcinoma relapse in 2 to 3 years [25, 150,161]. The treatment of relapsed androgen-independent prostate cancer remains a major oncological challenge. One of the approaches for improving the therapeutic response

and its duration could be based on combining LH-RH agonists or antagonists with peptides such as somatostatin analogs, bombesin/gastrin releasing peptide (GRP) antagonists, or GH-RH antagonists, which inhibit prostate cancers by interfering with the action, secretion, signal transmission, or receptors of endogenous growth factors such as IGF-I, IGF-II, and EGF [72,73,97,150]. Because the receptors for LH-RH, somatostatin, and bombesin/GRP are found in biopsy samples of human prostate cancer and various human prostate cancer lines, recently developed cytotoxic analogs of these peptides [118,157] could be used in the management of patients with advanced prostatic carcinoma who relapsed androgen ablation.

### *2.6. Cytotoxic analogs of LH-RH in experimental prostate cancers*

Receptors for LH-RH have been found in human prostate cancer samples [59,134], in androgen-sensitive LNCaP and androgen-independent DU-145 human prostate cancer cell lines [29,100,134], as well as in the Dunning R-3327-H and R-3327-AT-1 rat prostate cancer models [75]. Recent investigation of a large number of specimens of human prostate adenocarcinomas showed that 86% of cancers exhibited high affinity binding for LH-RH and expressed mRNA for LH-RH receptors [59]. The expression of specific LH-RH receptor in a high percentage of human prostate cancers [59] provides a rationale for the development of methods for therapy of this malignancy based on targeted cytotoxic LH-RH analogs.

Extensive investigations with new cytotoxic analogs of LH-RH were carried out in various models of prostate cancer. In the initial study, the effects of cytotoxic analog of LH-RH, AN-207, were evaluated in rats bearing hormone-dependent Dunning R-3327-H prostate carcinomas that express LH-RH receptors [75]. AN-207 was administered i.p. three times at a dose of 50 nmol/kg. After 5 weeks of treatment with a total dose of 150 nmol/kg AN-207, the prostate tumors regressed to approximately one-half of their initial volume while tumors in the control group continued to grow [75]. All rats injected with radical AN-201 died with signs of general toxicity. This work showed that the cytotoxic LH-RH analog AN-207 is much less toxic than its antineoplastic radical AN-201 and significantly more active in inhibiting prostate tumor growth [75].

In another study, we investigated the effect of cytotoxic LH-RH analog AN-207 on the growth of LH-RH receptor-positive PC-82 human prostate cancer xenografted into nude mice [90]. Analog AN-207, radical AN-201, and carrier [D-Lys$^6$]LH-RH were injected i.v. once at doses of 200 nmol/kg. Eight weeks after administration of cytotoxic analog AN-207, there was a major reduction in tumor volume and tumor burden and a decrease in serum PSA levels as compared with controls. Cytotoxic radical AN-201 caused no reduction in tumor volume and killed 40% of mice due to toxicity. Injection of AN-207 resulted in a fall in white blood count and in platelet count after 1 week, but the decrease in platelet count and white blood count was no longer significant 2–3 weeks after treatment [90]. A greater antiproliferative action and lower toxicity of AN-207, as compared with its radical AN-201, could be attributed to a more selective delivery of analog AN-201 to PC-82 tumor cells. After additional studies, it might be possible to use cytotoxic LH-RH analog AN-207 for the treatment of advanced prostate cancer after the relapse. Because of the presence of receptors of LH-RH on a high percentage of prostate cancers, targeted chemotherapy with cytotoxic LH-RH analogs should be more efficacious and less toxic than systemic chemotherapeutic regimens and might permit an escalation in doses.

In addition, cytotoxic analogs of LH-RH might also be indicated for primary therapy of patients with advanced prostate cancer, thus extending the oncological uses of LH-RH analogs from current palliation toward an eventual cure. It is also possible that repeated administration of cytotoxic analogs may totally eradicate some cancers.

### *2.7. Other cancers*

Much evidence suggests that exocrine pancreatic carcinomas and colorectal carcinomas may be sensitive in part to sex steroids [150]. Our work showed that tumor growth of BOP-induced pancreatic cancer in hamsters can be inhibited by treatment with agonist [D-Trp$^6$]LH-RH or the antagonist Cetrorelix [170,171], but clinical trials with LH-RH agonists in patients with pancreatic cancer were inconclusive or unsuccessful [45,51].

Because sex steroids and growth factors may play a role in promoting the transformation and/or proliferation of kidney neoplasms, we tested LH-RH antagonist Cetrorelix for its effects on the growth of the CAKI-I renal adenocarcinoma cell line xenografted into nude mice [74]. After 4 weeks of treatment, tumor volume in animals receiving these analogs was significantly decreased. Cetrorelix reduced serum testosterone [74] and the number of receptors for EGF on CAKI-I tumors. These peptide analogs should be considered for the therapy of patients with metastatic or recurrent renal adenocarcinoma cell and clinical trials are in progress.

## Acknowledgments

This article is dedicated to the memory of Geoffrey W. Harris, C.B.E., F.R.S, Dr. Lee's Professor of Anatomy in the University of Oxford, who established the basic theories of hypothalamic control of the pituitary gland. A few months before his untimely passing, he had the satisfaction of seeing his brilliant concepts and life's work confirmed by the isolation and identification of LH-RH.

Some original experimental work described in this paper was supported by the Medical Research Service of the

Veterans Affairs Department, an award from CaP CURE (Association for the Cure of Prostate Cancer), and grants from ASTA Medica to Tulane University School of Medicine. Some early experimental studies originating in this laboratory and cited in this review were also supported by U.S. Public Health Service Grants DK-07467, AM-09094, CA-40003, CA-40077, and CA-40004. I am grateful to various collaborators, scientists, and clinicians for important contributions on LH-RH and its analogs cited in this review.

It is our hope that the material covered will be a source for learning and a stimulus for further research and development of new therapeutic approaches. We would feel richly rewarded if our work could even in a small measure contribute to better methods for treatment of cancer and other diseases.

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Human Reproduction Update 2000, Vol. 6, No. 4 pp. 322–331 

# The LHRH antagonist Cetrorelix: a review

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**In those clinical situations in which an immediate and profound suppression of gonadotrophins is desired, LHRH agonists have the disadvantage of producing an initial stimulatory effect on hormone secretion. Therefore, the use of GnRH antagonists which cause an immediate and dose-related inhibition of LH and FSH by competitive blockade of the receptors is much more advantageous. One of the most advanced antagonist produced to date is Cetrorelix, a decapeptide which has been shown to be safe and effective in inhibiting LH and sex-steroid secretion in a variety of animal species and in clinical studies as well. Clinical trials in patients suffering from advanced carcinoma of the prostate, benign prostate hyperplasia, and ovarian cancer are currently in progress and have already shown the usefulness of this new treatment modality. In particular, the concept that a complete suppression of sex-steroids may not be necessary in indications such as uterine fibroma, endometriosis and benign prostatic hyperplasia represents a promising novel perspective for treatment of these diseases. Following completion of phase III trials in controlled ovarian stimulation for IVF regimens, Cetrorelix was given marketing approval and, thus, became the first LHRH antagonist available clinically.**

*Key words:* benign prostatic hyperplasia/cancer treatment/GnRH antagonist/gonadotrophins/ovarian stimulation

## TABLE OF CONTENTS



## Introduction

Analogues of the hypothalamic hormone, luteinizing hormone-releasing hormone (LHRH), which controls the secretion of the gonadotrophins, LH and FSH, from the anterior pituitary gland, belong to the standard medical armament for interfering with sex hormone production. The peptide hormone LHRH was isolated from hypothalamic extracts and its amino acid sequence was established by Schally *et al.* (1971), who also succeeded in synthesizing the hormone.

Replacement or deletion of different amino acids within the LHRH molecule resulted in the discovery of LHRH agonists, which possess an increased potency for the liberation of gonadotrophins. When agonistic analogues are applied continuously, following an initial stimulatory action, the opposite effect occurs, namely, an inhibition of gonadotrophin and sex-steroid secretion. The mechanism of this effect is based on a desensitization of the gonadotrophic cells and a down-regulation of pituitary receptors leading to a selective medical hypophysectomy. Several LHRH agonists are currently available and are used for the treatment of prostate and breast cancer, endometriosis and female infertility (Mansfield *et al.*, 1983; Lemay *et al.*, 1984; Schally and Redding, 1987). However, situations where an immediate and dose-dependent suppression of the gonadotrophins is required, the drawback of the agonists is in their initial stimulatory effect on hormone secretion and the relatively long period (2–3 weeks) of chronic exposure before complete suppression can occur. Hence, antagonists which can produce an immediate and dose-related inhibition of gonadotrophin release by competitive blockade of the receptors is more desirable (Figure 1).

Several groups headed by Schally and Rivier began synthesis of antagonistic analogues of LHRH >20 years ago. However, an early generation of LHRH antagonists was too lipophilic and subsequent generation not suitable for clinical use because of oedematogenic side effects caused by histamine release (Schmidt *et al.*, 1984; Hahn *et al.*, 1985).

Since then, major improvements have been achieved by the synthesis of LHRH antagonists incorporating further amino acid substitutions (Bajusz *et al*, 1988; Rivier, 1993). A modern

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antagonist which is devoid of oedematogenic effects is Cetrorelix, a decapeptide which has been shown to be safe and effective in inhibiting the secretion of gonadotrophins in a variety of species including man (Szende *et al.*, 1990; Behre *et al.*, 1992; Weinbauer *et al.*, 1993).

In this review, both the pharmaco–toxicological and the clinical results obtained with Cetrorelix will be summarised, including the indication of controlled ovarian stimulation for assisted reproduction techniques for which Cetrorelix (Cetrotide®; Asta Medica AG, Frankfurt, Germany) has recently been approved in Europe, thus making it the first GnRH antagonist to be marketed worldwide.

## Summary of preclinical results

### *Chemistry*

Cetrorelix is a decapeptide which was originally synthesized at Tulane University, New Orleans, USA, Bokser *et al.* (1990). Cetrorelix has a highly modified LHRH sequence, comprising 10 amino acids, five out of which are in a non-natural D-configuration (Table I). The C- and N-terminal protecting groups (acetyl, amide) provide stability and are mandatory for complete antagonistic activity. Cetrorelix was also investigated in terms of physico–chemical parameters, e.g. adsorption to surfaces and peptide aggregation. The tendency for aggregation and gel formation, as well as adsorption phenomena in general, were reduced by handling the peptide in a properly acidified aqueous solution for product transfer before lyophilization (Reissmann et al., 1994).

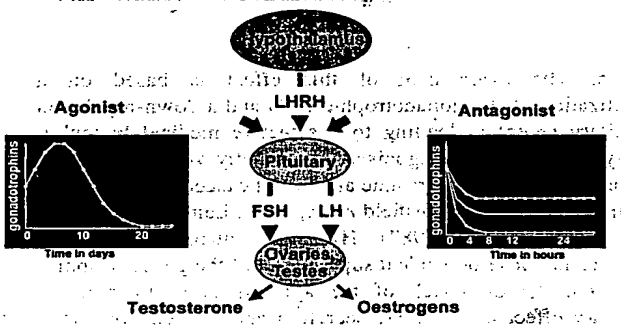


**Figure 1.** Luteinizing hormone-releasing hormone (LHRH) analogues – mode of action.

### *Stability*

Peptides are subject to hydrolysis, oxidation, photo decomposition and enzymatic proteolysis among other processes. The stability of Cetrorelix in aqueous solution was investigated in the pH range 1.0–13.0. The peptide was found to be surprisingly stable for a period of 21 days at room temperature at pH 7.0 with significant decomposition at higher or lower pH values. It is resistant to oxidation with $H_2O_2$ under neutral conditions, but decomposes up to 15% within 30 min at 100°C at pH 7.0. When stored at refrigerator temperature (2–8°C) the substance as well as the lyophilisate are stable for at least 3 years. The reconstituted solution (lyophilisate dissolved in water for injection) is stable for at least 2 days when stored at room temperature (20°C). Cetrorelix is highly resistant to degrading enzymes, e.g. chymotrypsin, pronase and nargase (subtilisin), for up to 50 h at 37°C (Reissmann *et al.*, 1994). This is in sharp contrast to potent LHRH agonists which face almost complete degradation within several hours. The proteolytic stability of Cetrorelix is underlined in comparison with a diastereomeric analogue comprising L-configured citrulline in position 6, instead of D-citrulline as in Cetrorelix. This analogue is highly sensitive to degradation and lacks biological activity, probably due to this imminent enzymatic instability (Pinski *et al.*, 1995).

### *LHRH receptor binding*

Binding affinities of Cetrorelix and the agonist (D-TRP$^6$)-GnRH to membrane receptors on cells from male rat pituitary glands were estimated using labelled GnRH (Fekete *et al.*, 1989). Results indicated that LHRH binds to two classes of membrane receptors on pituitary cells, one with low, the other with high affinity. LHRH is displaced by Cetrorelix from both receptors, which has an affinity constant ~5 times higher for the first and 1.4 times higher for the second receptor class.

These data were confirmed in a mouse fibroma cell line model which was transfected with the gene for the human LHRH receptor protein and showed a stable expression (Beckers *et al.*, 1995). The binding and affinity of Cetrorelix to this receptor was

**Table I.** Sequence of amino acids in luteinizing hormone-releasing hormone (LHRH) and Cetrorelix

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| GnRH | Glu | His | Trp | Ser | Tyr | Gly | Leu | Arg | Pro | Gly-$NH_2$ |
| Cetrorelix | D-Nal | D-Phe | D-Pal | 4 | 5 | D-Cit | 7 | 8 | 9 | D-Ala |

Molecular weight = 1431.07.

**Table II.** Dissociation constants of luteinizing hormone-releasing hormone (LHRH) analogues for the human LHRH receptor

| Peptide | LHR | Cetrorelix | Antide | Buserelin |
|---|---|---|---|---|
| KDa × $10^9$ (mol/l) | 1.47 ± 1.23 | 0.19 ± 0.03 | 0.36 ± 0.13 | 0.30 ± 0.12 |

determined in comparison to Antide, an antagonist which has already been tested in phase I clinical trials, and to the agonist buserelin (Suprefact® Hoechst AG, Frankfurt, Germany). Cetrorelix has a binding affinity ~20 times higher than the native LHRH and about twice as high as the agonist, buserelin, or the antagonist, antide (Table II).

### *Preclinical safety evaluation*

In the past, the problems in the development of LHRH antagonists included the property to induce a systemic liberation of histamine. The introduction of D-Cit in position 6 of an antagonistic decapeptide results in a less intense histamine release, as demonstrated in rat mast cells in an in-vitro assay system (Bajusz *et al.*, 1988). Subsequently, the histamine releasing activity of Cetrorelix was determined during the safety pharmacological evaluation at first in this assay system. The results indicated that the $ED_{50}$ value obtained for Cetrorelix was >1000-fold higher than the pharmacological effective plasma concentrations and, therefore, can be regarded as being of no clinical relevance. However, in view of the high variability, this assay has a limited predictivity if the test compounds to be compared are not evaluated within the same assay and if they have different solubilities in the assay medium. Therefore, in-vivo experiments appeared to be more suitable as a test method. Injections of Cetrorelix into rats at a dose of 1.5 mg/kg showed no oedematous reaction. In addition, the examination of systemic effects revealed that with i.v. injections at doses of 1 and 4 mg/kg no apparent changes in respiratory and cardiovascular functions were observed. In both pharmacological and long-term toxicological safety studies in rats and dogs, Cetrorelix exerted no systemic side-effects. A variety of organ changes, related to the pharmacodynamic effects did not show any progressive properties or were morphologically and/or functionally reversible after cessation of treatment. No direct target organ toxicity was found in acute, subacute or chronic toxicity experiments. No contact sensitizing properties, teratogenic potential, or influence on the early embryonic development and implantation were detected at clinically relevant doses. Mutagenicity tests were unequivocally negative for genmutagenic and chromosomal aberration endpoints.

### *Animal experiments*

#### *Effects on LH, FSH and testosterone concentrations*

In most of the experiments performed on animals, Cetrorelix was tested alone or in comparison with GnRH agonists, since a standard antagonist did not exist. In order to assess its hormone suppressive potency, treatment with Cetrorelix was carried out in mice, rats and monkeys. In what follows, some selected results are described illustrating the mode of action and efficacy of Cetrorelix. In order to determine whether Cetrorelix also competes with LHRH agonists *in vivo* for the same binding sites on the pituitary cells, investigations were conducted as to whether the antagonist-induced suppression of LH can be overcome by the administration of the agonist Decapeptyl® (D-$Trp^6$-GnRH). The results showed that a stimulation of LH was indeed achieved by Decapeptyl during the suppressive treatment with Cetrorelix. This stimulation was of lower amplitude than in the control group, but was consistent at different time points indicating that both compounds compete for the LHRH binding sites. When Cetrorelix was given shortly before the agonist, the stimulated LH response was significantly lower and could even be nullified (Pinski *et al.*, 1992). Under these conditions, the binding sites available for the agonist were obviously not sufficient for the induction of gonadotrophin release. The pituitary receptors occupied by Cetrorelix appear to be identical to those required for D-$TRP^6$-binding. Based on these results, it can be concluded that Cetrorelix is a LHRH antagonist which specifically and competitively binds to high-affinity GnRH receptors, thereby inhibiting the release of gonadotrophins from the pituitary gland.

The castrated male rat is a commonly used model which allows the measurement of even low suppressive effects on gonadotrophins, since increased LH and FSH plasma concentrations are present due to the absence of a negative feed-back of testosterone. In these animals a dose-dependent suppression of LH was achieved and a single dose as low as 2.5 µg/rat (~10 µg/kg) significantly decreased plasma LH concentrations immediately after a s.c. injection. The nadir was reached after four h with a fall in LH concentrations of >80%, with serum LH returning to normal after 24 h. Further increase in dosage prolonged the duration of LH suppression (Bokser *et al.*, 1991). The efficacy of these low doses already indicates the high suppressive potency of Cetrorelix, which can also be expected in intact animals. In intact female rats the dose of 2.0 µg/rat was sufficient to completely inhibit the ovulation and even lower doses significantly reduced the rate of ovulation. All doses were devoid of macroscopically visible side-effects; furthermore, the recovery of hormone values and gonadal function after prolonged treatment was confirmed (Bokser *et al.*, 1990).

The gonadotrophin suppression induced by Cetrorelix was also tested in the sub-human primate *Macaca fascicularis*. As had been shown previously in rats, an immediate suppression of LH concentrations could be observed in male castrated monkeys as well. There were no differences between the treatment groups consisting of single doses of 250, 625 and 1250 µg/kg Cetrorelix. The nadir was reached at ~12 h post-injection and surprisingly, the LH concentrations remained suppressed for at least 96 h. Even at the lowest dose, there was no tendency for a LH rebound within this time interval, thus indicating again the high LHRH antagonistic potency (Weinbauer and Nieschlag, 1993). A dose of 225 µg/kg given daily for 14 days to intact monkeys produced a continuous suppression of testosterone to castration concentrations (Weinbauer *et al.*, 1993). Haematology and clinical chemistry parameters showed no pathological alterations. During prolonged treatment with a dose of 450 µg/kg s.c. daily for 7 weeks, a continuous suppression of LH and testosterone was induced in these intact monkeys. Additionally, starting at week 2 of treatment serum concentrations of inhibin were suppressed and testicular volume decreased; following continuous Cetrorelix injections, all animals became azoospermic (Weinbauer *et al.*, 1994). After termination of treatment all effects were reversible.

Investigations have shown that FSH concentrations are not affected by Cetrorelix as strongly as LH during short-term treatment, which might be due to differences between bioactive and immunoactive FSH, differential regulation of LHβ and FSHβ subunit expression and/or the prolonged plasma half-life of FSH (Matikainen *et al.*, 1992). In rats, the

pituitary LH and FSH content was not altered by the treatment with Cetrorelix, indicating lack of effect on the synthesis of gonadotrophins by the gonadotroph cells of the anterior pituitary gland (Ayalon *et al.*, 1996). In agreement with these results, single high doses resulted in an immediate arrest of the oestrous cycle in rats with the duration being dose-dependent (Reissmann *et al.*, 1996). In-vitro tests revealed that plasma concentrations achieved in these experiments do not interfere with epidermal growth factor (EGF)-stimulated human granulosa cell proliferation, which was only inhibited by concentrations that were ~100 times higher (Yano *et al.*, 1997). These results prove that Cetrorelix effectively and dose-dependently suppresses the secretion of gonadotrophins, especially LH, from the pituitary gland. As a result of this hormone-withdrawal, pre-ovulatory LH peaks can be inhibited. When given at sufficiently high doses, a cessation in reproductive function in female and male animals is observed, which is reversible after treatment termination.

## Summary of clinical results

### *Phase I studies*

Based on the preclinical efficacy and a favourable safety profile, clinical phase I studies were initiated in volunteers. In 15 phase I studies, including single and multiple s.c. injections as well as single i.v. infusions, Cetrorelix was administered to a total of 236 healthy subjects of both sexes (161 male and 75 female). The dose range tested for single doses was 0.25–20.0 mg s.c. The results of representative studies are summarized below.

The first administration of Cetrorelix to man was performed as single s.c. doses in healthy male volunteers (Klingmüller *et al.*, 1991; Behre *et al.*, 1992). Compared with the placebo group, the extent and duration of suppression increased in parallel with increasing doses. After the administration of 1.0 mg, a maximal testosterone suppression of 73% in comparison with baseline was seen 8 h after injection; the suppression was 80 and 91% after single doses of 2.0 and 5.0 mg respectively. By 48 h after the injection of the 5 mg dose, testosterone values were no longer different from those in the placebo group and reached serum concentrations in the lower normal range. As in animal studies, the suppression of FSH did not reach a statistical significance. Linear kinetics were found, with a calculated plasma t½ of 30 h after single doses of 5 mg Cetrorelix.

Single dose administration of Cetrorelix to healthy pre-menopausal female subjects was performed in view of the planned clinical use of Cetrorelix in IVF programmes. Following single doses of 3 and 5 mg administered between days 6 and 10 of the menstrual cycle, serum LH, FSH and oestradiol decreased immediately (Leroy *et al.*, 1994). A nadir was reached 24 h after injection with a reduction of 56 ± 19, 29.5 ± 16 and 85 ± 17% (48 h) compared with baseline respectively. No significant differences with regard to the extent of suppression were seen between the two different doses. The LH surge was postponed in all cases, occurring 6–17 days after the Cetrorelix injection. When Cetrorelix was administered during the late follicular phase during which time plasma oestradiol concentrations were >150 pg/ml, spontaneous LH surges were also postponed in all women. Again, the suppression of FSH was less pronounced, giving early reason for speculations that a reduced stimulation procedure could be applied during controlled ovarian stimulation cycles.

Single i.v. administration of Cetrorelix to healthy men was performed in order to determine pharmacokinetics, absolute bioavailability, pharmacodynamic effects, safety and tolerability. Six healthy male subjects randomly received single doses of 3 mg Cetrorelix i.v. and s.c. with a wash-out period of 21 days between each single administration. The extent of suppression was the most pronounced for testosterone, reaching mean decreases from baseline of 93% (i.v.) and 95% (s.c.). Compared with the baseline, LH was reduced by 82% (i.v.) and 80% (s.c.), whereas FSH values were influenced less, as seen by a decrease from baseline of 41 and 49% after i.v. and s.c. administration respectively (Hermann *et al.*, 1996).

Multiple dose administration to healthy men ranged from 0.25 to 10 mg given daily for 7, 8 or 14 days. During daily doses for 8 days (Behre *et al.*, 1994), a dose-dependent suppression of gonadotrophins and testosterone was found, but interestingly only a dosage as high as 10 mg/day was able to maintain castration concentrations of testosterone over the period of administration. When lower doses were used, an increase of testosterone concentrations was found between days 2–4 of treatment. Therefore, based on results from animal studies and pharmacokinetic considerations, an investigation was conducted to determine if the suppression of LH, FSH, and testosterone can be achieved by an initial high-dose and thereafter be maintained by continued low-dose injections. A loading-dose schedule using 10 mg/day for 5 days followed by a maintenance dose of 1 mg/day was then tested in male volunteers (Behre *et al.*, 1997) which resulted in a continuous suppression of testosterone to castration range. In addition, it was found in these studies that treatment with Cetrorelix induced a pronounced and reversible reduction (~40%) of the prostatic volume within 2 weeks of treatment.

Female volunteers were observed during three consecutive menstrual cycles consisting of pre-treatment, treatment and a post-treatment control cycle. Subjects received 3 mg Cetrorelix s.c. daily for 1 week with the first injection administered on day 8 of the individual cycle. By 24 h after the first application, LH was strongly suppressed and oestradiol reached post-menopausal values. The mean duration of the suppressive effects of Cetrorelix after the last Cetrorelix injection compared to baseline values was 13.0 days for LH, 9.4 days for FSH and 14.6 days for oestradiol. In the nadir (day 15) LH was reduced to 16.1% and FSH to 63.5% of the respective baseline values and oestradiol to post-menopausal values (Gonzalez-Barcena *et al.*, 1994b; Sommer *et al.*, 1994). After termination of treatment, an LH surge followed by post-ovulatory progesterone values was found in all women. Pharmacokinetic analyses of a study in female volunteers receiving daily doses of 0.25, 0.5 and 1 mg from cycle days 3 to 16 revealed dose-linearity and a single dose of 3 mg resulted in a plasma half-life of ~8 h after iv.-injection and 25 h after s.c. injection with a bioavailability of 92% (Hermann *et al.*, 1996).

### *Tolerability in phase I studies*

Since the histamine releasing potential and subsequent severe local and systemic side-effects, e.g. oedema, anaphylactoid

reactions had been previously recognized with the use of former LHRH antagonists, a intradermal skin test was usually performed prior to a systemic administration of therapeutic doses. Thus, in most of the initial studies an intracutaneous test with 10 μg Cetrorelix was performed which would lead to exclusion of a patient in case of severe local and/or systemic allergic reactions. However, no systemic adverse reactions were reported in any of these trials. Mild local reactions were reported with symptoms such as redness at the injection site, but these reactions were mild and transient. During further investigations, 25 healthy volunteers of each sex received five consecutive intracutaneous tests (10 μg Cetrorelix). It became apparent that the intracutaneous test did not result in reproducible effects on the skin and was not at all predictive of the occurrence of local or systemic side-effects in subsequent s.c. treatments. The most frequent local reaction following s.c. single doses was redness/erythema and was classified as being of slight to moderate intensity and subsided spontaneously and completely within minutes. The inter- and intra-subject (day-to-day) variability of the erythema size was remarkably high. There was no evidence that the frequency of local reactions depends on the number of consecutive administrations. Local reactions at the site of injection occurred independently of dose or sex and also occurred after placebo (Hermann *et al.*, 1996). Other side-effects were based on the pharmacodynamic action of Cetrorelix, i.e. suppression of testosterone or oestradiol, and consisted of a decreased libido and hot flushes. Laboratory parameters showed no alterations following single dose, whereas time and dose dependent increases of high-density lipoprotein (HDL) cholesterol were observed during multiple dose treatment. No significant change was seen in serum concentrations of low-density lipoprotein (LDL) cholesterol or triglycerides (Behre *et al.*, 1994).

### *Phase II studies in different indications*

#### *Controlled ovarian stimulation for assisted reproductive techniques*

For ovarian stimulation, either human menopausal gonadotrophin (HMG) or, more recently, recombinant (r)FSH is used followed by ovulation induction with human chorionic gonadotrophin (HCG), which is injected when a sufficient number of mature follicles are present. Thereafter, assisted reproductive techniques, such as IVF or intracytoplasmic sperm injection (ICSI) are used in order to obtain embryos for replacement in the uterine cavity. The classical stimulation procedure with gonadotrophins has the disadvantage of an unpredictable ovarian reaction and the occurrence of a premature LH surge, caused by the positive feedback of rising oestradiol in up to 25% of the cases (Schmutzler and Diedrich, 1990). The high LH concentrations during this surge may have deleterious effects on the quality of oocytes and the increasing progesterone concentrations may have a negative effect on the endometrium, both reducing pregnancy rates and thus leading to the cancellation of the treatment cycle. LHRH agonists were added to the stimulation protocols to prevent premature LH surges by inducing a suppression of endogenous LH, thereby achieving a reduction in the frequency of premature luteinization to ~2%. Presently, the so-called long protocol in which the treatment with agonists starts at least 14 days prior to stimulation with gonadotrophins, appears to be the most effective procedure. However, there are some disadvantages of this treatment schedule: (i) a long treatment period before the suppression of gonadotrophins occurs and ovarian stimulation with exogenous gonadotrophins can be started (~14 days in the long-protocol); (ii) a relatively long exposure to hormonal medication; (iii) a strong suppression of both gonadotrophins and oestradiol resulting in the occurrence of hormone-withdrawal symptoms, e.g. hot flushes, and the requirement for high doses of HMG/FSH for stimulation; and (iv) as a result of the induced down-regulation of the receptors and a depletion of gonadotrophin storage vesicles, a recovery period is necessary for the restoration of pituitary responsiveness which might contribute to the requirement of luteal phase support.

New treatment modalities are now available based on the use of Cetrorelix in these procedures, since because of the immediate suppression of gonadotrophins the unwanted stimulatory phase produced by the LHRH agonists can be avoided and the duration of treatment duration can be significantly reduced by administration of Cetrorelix only during the period of increased risk for premature LH surges.

Phase II clinical trials including 294 patients were conducted to investigate the efficacy and safety of Cetrorelix (Cetrotide) in controlled ovarian stimulation for assisted reproduction techniques using HMG, since rFSH had not been registered in Europe when the study was initiated. Two different dose regimens of Cetrorelix were applied consisting of multiple doses of 3, 1, 0.5, 0.25 and 0.1 mg/day starting on cycle day 5 or 6 until and including the day of HCG administration, and a single or dual dose of 5, 3 and 2 mg given primarily on stimulation day 7. For both types of treatment, a minimal effective dose for the prevention of premature LH surges (defined by LH ≥10 IU/l and progesterone ≥1 ng/ml), was determined (Felberbaum and Diedrich, 1999) (Figure 2).

Diedrich *et al.* (1994) included a total of 20 patients with primary or secondary tubal sterility undergoing controlled ovarian stimulation. In all, 15 patients were treated with 3 mg of Cetrorelix (Cetrotide) daily s.c. starting on day 7 of the menstrual cycle until the application of HCG. Since no endogenous LH surge was seen, five additional patients were treated with a dose of 1 mg/day of Cetrorelix using the same treatment schedule; however, once again no LH surge was observed. Following the first Cetrorelix dose, LH

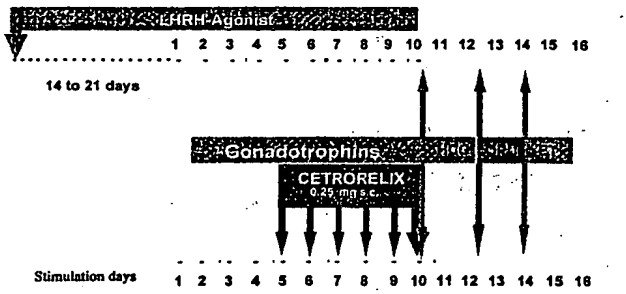


**Figure 2.** Cetrorelix in controlled ovarian stimulation for assisted reproduction techniques: treatment schedule for daily 0.25 or single 3 mg dose. HCG = human chorionic gonadotrophin; OPU = oocyte retrieval; ET = embryo transfer.

values fell immediately and this suppression was evident for both the 3 mg/day dose and 1 mg/day dose of Cetrorelix. Mean oestradiol concentrations were unaffected and underwent normal changes indicative of continuous follicular development. The quantity and quality of oocytes was comparable with the use of the long protocol with agonists as applied in the corresponding institution. In all patients the oocytes could be collected and fertilized (61.5% fertilization rate), and embryo transfer was subsequently performed in all cases. As could be expected from the results of phase I trials in women, the number of gonadotrophin ampoules needed could be reduced to 27, compared with 35–40 ampoules with the long protocol with agonists in this centre. The responsiveness of the pituitary was also examined in this study by performing a LHRH test 3 h before ovulation induction. Administration of 25 μg of LHRH and measurement of the LH release 30 min later revealed that a mean increase in serum LH was 10 mIU/ml for the 3 mg group, while the average maximum in the 1 mg group was ~32.5 mIU/ml, thus indicating a preserved pituitary function (Felberbaum *et al.*, 1995).

In subsequent studies (Albano *et al.*, 1997) a further reduction in the amount of Cetrorelix to daily injections of 0.5, 0.25 and 0.1 mg was evaluated in a total of 90 patients. In this study a daily s.c. dose of 0.25 mg of Cetrorelix proved to be the minimal effective dose to prevent premature LH surges and to obtain good quality of oocytes (Figure 3). Analysis of Cetrorelix in serum during the entire administration period and in follicular fluid obtained at the day of oocyte retrieval showed concentrations at or below the detection limit (0.3 ng/ml). On the day of embryo transfer, no plasma concentrations of Cetrorelix were detected.

In order to simplify the stimulation protocol further, the suitability of a single dose injection was evaluated. Frydman *et al.* included 17 patients in a study to assess a duration of action of a single 5 mg dose injected when oestradiol concentrations were between 150–200 pg/ml per follicle >14 mm (Olivennes *et al.*, 1994). A second dose was injected 48 h later if the triggering of ovulation was not decided upon. As a result, six patients were injected with a single dose and 11 patients received two injections, the mean day of the first injection being day 9.6. At 24 h after injection a mean decrease in LH to 0.2 mIU/ml (5.5 mIU/ml baseline) occurred and hence no premature LH surge was observed until the day of ovulation induction which was performed on cycle day 11.7.

Thereafter, 11 women were included in a IVF protocol and received the single dose of 3 mg Cetrorelix on day 8 of the menstrual cycle. In eight patients a single injection was sufficient and ovulation induction was performed on cycle day 11 ± 1. In the remaining three patients a second injection of 3 mg Cetrorelix was necessary 72 h after the first dosing and ovulation was triggered on cycle day 12.3 ± 0.6. Subsequently, it was confirmed that the 3 mg dose represents the minimal effective dose for preventing premature LH surges (Figure 4) in the majority of patients when given on cycle day 8 (Olivennes *et al.*, 1995, 1998). Overall, 294 patients were included into the phase II development of Cetrorelix in controlled ovarian stimulation for assisted reproduction, in which a pregnancy rate per embryo transfer of 30% was achieved.

In order to confirm these results, a phase III trial programme was initiated including three multicentric, multinational studies. Multiple doses of 0.25 mg/day of Cetrorelix starting on stimulation day 5 or 6 were tested. Buserelin nasal spray (0.6 mg/day) starting on pre-cycle day 20 was given to a control group of patients. In addition, the use of a single dose of 3 mg Cetrorelix on stimulation day 7 was evaluated as well as a single dose of 3.75 mg triptorelin depot in the control group. The results of these studies are not yet published, but it is expected that with respect to number of follicles, oocytes retrieved, fertilization rate and pregnancy rates, the results with Cetrorelix will be comparable to those obtained in patients under LHRH agonist treatment. This assumption is based on preclinical findings showing that Cetrorelix does not affect steroid biosynthesis of granulosa–lutein cells or growth-factor induced granulosa cell proliferation (Yano *et al.*, 1997) in an in-vitro setting. Furthermore, no concentrations of Cetrorelix can be measured in follicular fluid or plasma at the time of oocyte retrieval.

The prevention of premature LH surges with Cetrorelix offers the possibility of using different options to induce final oocyte maturation and ovulation. Due to the preserved pituitary responsiveness to LHRH under Cetrorelix treatment (Felberbaum *et al.*, 1995), the idea was conceived of administering a single injection of an LHRH agonist or recombinant LH (Sills *et al.*, 1999) for the induction of

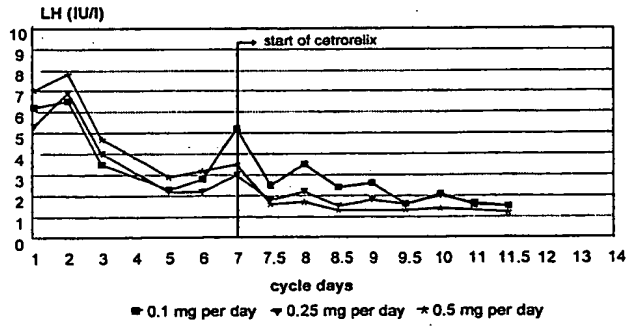


Figure 3. Cetrorelix in controlled ovarian stimulation for assisted reproduction techniques: mean LH concentrations during treatment with daily s.c. doses of 0.1, 0.25 and 0.5 mg.

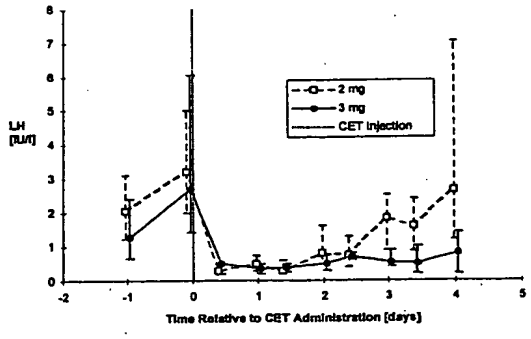


Figure 4. Cetrorelix (CET) in controlled ovarian stimulation for assisted reproduction techniques: mean LH concentrations after treatment with single s.c. doses of 2 and 3 mg.

ovulation, thus avoiding the administration of HCG. Therefore, attempts were made to induce ovulation by a single s.c. administration of the LHRH agonist, Triptorelin, in five patients treated according to the single dose regimen in a controlled ovarian stimulation programme (Olivennes *et al.*, 1996). Following the injection of Triptorelin, an LH surge was observed in all five patients and 12 h later mean plasma LH concentrations had increased from 1.3 ± 1.0 to 56.3 ± 40.0 IU/l, followed by a significant rise of plasma progesterone concentrations to 17.27 ± 4.12 ng/ml 72 h after Triptorelin injection. Thus, Cetrorelix treatment allows ovulation induction by LHRH or one of its agonists instead of HCG, which could be beneficial in patients at high risk of ovarian hyperstimulation syndrome (OHSS) and those suffering from polycystic ovarian disease (PCOD) (Felberbaum and Diedrich, 1999). Clinical studies comparing the use of HMG versus rFSH in controlled ovarian stimulation for assisted reproduction techniques with Cetrorelix are ongoing. In addition, the concept of the so-called 'natural cycles' which include a minimal ovarian stimulation can be applied with the use of Cetrorelix (Rongières-Bertrand *et al.*, 1999). In this study, Cetrorelix was given as a single sc. injection of either 1 or 0.5 mg in the late follicular phase of 44 cycles, when oestradiol values were 100–150 pg/ml. A mean of 4.7 ampoules of HMG were used for stimulation and treatment resulted in a successful inhibition of the LH surge in all available patients and in a pregnancy rate of 32% per transfer.

### *Uterine myoma*

Endometriosis and uterine fibroids are diseases dependent on oestrogens. In both conditions the therapeutic use of LHRH agonists is well established, but again the initial flare-up is undesirable and the necessity of achieving castration concentrations of oestradiol is questionable. Therefore, the potential advantage of a LHRH antagonist is obvious. Results of pilot trials with Cetrorelix indicate an effective reduction in myoma size, a shorter treatment period in comparison with LHRH agonists and indeed, shrinkage of the myoma without castration (oestradiol >50pg/ml) can be obtained.

An explorative study was conducted to investigate the efficacy and safety of Cetrorelix in the medical management of uterine leiomyomata (Gonzalez-Barcena *et al.*, 1997). Cetrorelix was administered to 18 pre-menopausal women with myomata who had been scheduled for hysterectomy. The initial dose of Cetrorelix was 5 mg s.c. twice daily for the first 2 days and thereafter 0.8 mg s.c. twice daily for at least 3 months until the day of surgery. The mean duration of treatment was 4.4 months. The mean baseline uterine volume was 395.4 ± 69.2 ml, and after 3 months 16 patients showed a progressive reduction in uterine volume to a mean of 230.8 ± 52.6 ml . All patients became amenorrhoeic under these conditions. After termination of treatment with Cetrorelix, a myomectomy was performed in 12 women. Further (unpublished) studies suggest that the reduction in size and vascularity of the uterus make surgical exstirpation of the myomata much more feasible. Promising results were also obtained in a clinical trial (Felberbaum *et al.*, 1998), which used a Cetrorelix depot formulation, and showed that a complete suppression of oestradiol might not be necessary to obtain a maximum reduction of myoma size already after a 4 week treatment period. Preliminary results from a ongoing multicentre study in Japan also indicate that a short treatment period and incomplete oestradiol suppression are sufficient for reducing the volume of uterine fibroids substantially within 4 weeks of treatment using a dose of 3 or 5 mg Cetrorelix once a week. Gynaecological studies with Cetrorelix have been summarized recently by Schally (1999a).

### *Ovarian cancer*

LHRH receptors are expressed in ~80% of ovarian cancers and there is experimental evidence that the growth of these receptor-positive cells can be inhibited by LHRH analogues (Emons and Schally, 1994; Srkalovic *et al.*, 1998). Since Cetrorelix was shown to be more active than agonistic compounds in some models of ovarian cancer (Shirahige *et al.*, 1994), a phase I/II clinical trial was started in patients with advanced disease after first-line chemotherapy. Patients showing progressive disease were treated with Cetrorelix in a dose of 10 mg s.c. daily. Presently, 17 patients are available for response; of these, three have shown a partial remission and four a stabilization of their disease (no change). In these patients the time to progression was between 125 and 196 days, whereas for all patients treated this value was 59 days. These results are very encouraging since 15 patients had progressive disease after third to fifth line therapies before starting Cetrorelix. Currently, patients are being treated according to a stratification consisting of platinum-resistant and platinum-sensitive groups (Emons *et al.*, 1999).

### *Benign prostatic hyperplasia (BPH)*

The standard therapy to achieve total testosterone suppression is orchidectomy with the occurrence of hormone-withdrawal symptoms such as hot flushes and impotence, which should be avoided in a benign disease such as BPH. Since total testosterone suppression might not be mandatory in BPH, theorectically it should be possible to apply a short-term treatment and produce the degree of testosterone suppression as low as necessary using an LHRH antagonist, e.g. Cetrorelix. Therefore, several studies have been performed including 114 patients who were treated with various dosages and dose schedules of Cetrorelix. These studies have shown the effectiveness of Cetrorelix as shown by an increase of peak urinary flow rate, a reduction of prostate volume and improvement in other BPH symptoms. The efficacy initially observed in uncontrolled pilot studies (Gonzalez-Barcena *et al.*, 1994a) was confirmed in a double-blind placebo-controlled study (Lepor *et al.*, 1997). In this trial patients received s.c. injections of Cetrorelix at a dose of 1 mg/day for 4 weeks. At the end of the treatment period a shrinkage of the prostate and a major improvement of urine flow were noted. Surprisingly, these effects were obtained although the administered dose of Cetrorelix suppressed the serum concentrations of testosterone only by ~50%. The improvement in BPH symptoms lasted for several months and was independent of prostate size at study entry. These results were substantially extended by Comaru-Schally *et al.* (1998), who treated patients with moderate to severe symptomatic BPH with Cetrorelix. In this study a significant (53%) reduction in urinary symptom score, a 46% improvement in the quality of life and increase in urinary flow-rate was found, although testosterone was only suppressed by 64–74% during maintenance therapy. During the follow-up, the effects

proved to be long-lasting with a decline in the international prostate symptom score (IPSS) of 72% still being present at study week 85. The fast onset and long duration of efficacy indicate that growth factors not yet identified may play a role in the mechanism of Cetrorelix action. Thus, beneficial clinical effects could be obtained without the negative consequences associated with a classical anti-androgen therapy. These results indicate that an intermittent treatment schedule, possibly in combination with an α-receptor blocker, could be elaborated in subsequent trials, thus opening the possibility of achieving a sustained improvement in symptoms using a few short-term treatment cycles per year.

### *Prostate cancer*

Several open label phase I/II studies in patients with advanced prostate cancer were performed using different dose schedules of Cetrorelix acetate (Gonzalez-Barcena *et al.*, 1994a, 1995, 1996; Ayalon *et al.*, 1996). In a first explorative study, six patients with biopsy-proven prostatic cancer (two stage C and four stage D2) received Cetrorelix daily in terms of s.c. injections of 0.5 mg twice daily for 6 weeks. The treatment resulted in an immediate fall of testosterone to subnormal concentrations, reaching a nadir after 6–12 h. After 6 weeks of therapy total serum testosterone was below castration concentration (2 nmol/l). At the end of treatment the values of acid prostatic, total acidic and alkaline phosphatases reached normal values in all patients, whereas PSA concentrations were in the normal range in three patients. After the first week of treatment, a significant decrease in bone pain, relief of urinary flow obstruction and reversal of signs of prostatism was observed. Subjective improvement continued during the following weeks and ultimately the patients no longer needed analgesics. A progressive decrease in prostate volume was obtained starting in the second week of treatment.

Since this dose regimen did not achieve castration concentrations of testosterone from the beginning, an initial loading dose schedule was applied (Gonzales-Barcena *et al.*, 1996; Ayalon *et al.*, 1996) which had been shown to be effective in a preceding Phase I study (Behre *et al.*, 1997). The results obtained with respect to dose finding for continuous castration are in accordance with these phase I results and showed that a loading dose of 10 mg daily for 2–5 days is required to achieve suppression of testosterone to castration concentrations in all patients from the beginning. Thereafter a daily dose of 1–2 mg is effective in maintaining castration concentrations. Despite the fact that not all dose schedules tested resulted in castration concentrations of testosterone from the beginning, the treatment proved to be clinically effective as evidenced by reduction of serum PSA, regression of metastatic lesions and fast improvement of disease-related symptoms, e.g. bone pain, paraesthesia and paraplegia and hence, no co-medication with anti-androgens is required in symptomatic patients.

## Conclusions

The decapeptide Cetrorelix has been extensively characterized as a potent antagonist of LHRH in various in-vitro animal models. The hormone-suppressing effects are dose-dependent and can be induced immediately after the start of the administration thereby avoiding the 'flare-up effect' seen with the LHRH agonists. All treatment related effects are reversible and no teratogenic, mutagenic or contact sensitizing properties were found in toxicological studies. The human results on the LHRH antagonist Cetrorelix clearly demonstrate its safety profile and potential usefulness in the clinic. Therefore, Cetrorelix is also suitable for the use in controlled ovarian stimulation for assisted reproduction techniques and treatment of benign conditions including leiomyoma, endometriosis and BPH.

In patients undergoing controlled ovarian stimulation an excellent efficacy and safety profile was shown. Prevention of LH surges with Cetrorelix offers the possibility of using different options for the development of follicles, the induction of final oocyte maturation and ovulation. This in turn permits the simplification of procedures and reduces the risk and duration of exposure of patients to hormonal treatment. This could be beneficial in patients at high risk of OHSS and those suffering from PCOD. The results from clinical phase IV studies are expected to further substantiate the advantages of Cetrorelix compared with the use of LHRH agonists for this indication.

Preliminary results obtained following Cetrorelix treatment of patients with leiomyoma show a rapid reduction of uterus and myoma volumes within a 4 week treatment period. Therefore, the LHRH antagonist Cetrorelix should also be indicated for the treatment of endometriosis. In both conditions the possible advantages of LHRH antagonists still have to be demonstrated in large-scale studies; however, based on the clinical efficacy already demonstrated for LHRH agonists, their benefits may be deduced.

All studies performed to date in BPH patients reveal that Cetrorelix is effective in this condition. A daily treatment for 4 weeks produces a clinically significant and long-lasting improvement in BPH symptoms for at least 3 months. It is important that this can be achieved without testosterone suppression to castration concentration and related side-effects. This indicates that Cetrorelix may be used for intermittent therapy in BPH based on 1–3 treatment cycles per year.

Because of a strong suppression of sex-steroids, Cetrorelix can be utilized for the treatment of hormone-dependent cancer such as prostate and ovarian cancer. Oncological studies with Cetrorelix have been summarized recently (Schally, 1999b). In prostate cancer patients, treatment with Cetrorelix proved to be clinically effective as evidenced by reduction in serum PSA, regression of metastatic lesions and fast improvement of disease-related symptoms, e.g. bone pain, paraesthesia and paraplegia. Since this antagonist successfully inhibits spermatogenesis, it might also be useful for the prevention of gonadal damage by cytostatic agents during chemotherapy or radiotherapy (Ataya *et al.*, 1988; Schally *et al.*, 1989; Meistrich, 1998).

Currently, daily injections of Cetrorelix are used, however, by increasing the dose new treatment schedules, e.g. 3 mg per week, can be applied in indications requiring treatment for several weeks as may be the case for uterine fibroids and BPH. For long-term treatment such as for various cancers, a depot formulation is desirable and different approaches towards a clinically suitable depot formulation are under evaluation.

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*Received on December 23, 1999; accepted on March 20, 2000*

Depot
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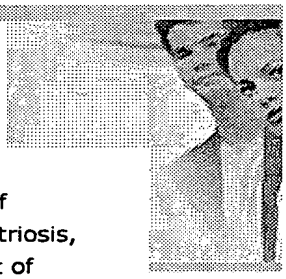

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Learn more about Lupron Depot by accessing complete prescribing information below and visiting the Lupron Web site.

Learn more about the following conditions:
Prostate Cancer
Endometriosis and Uterine Fibroids
Central Precocious Puberty

**Lupron Depot Prescribing Information**

**Urology**
Lupron Depot® - 4 Month 30 mg, Lupron Depot® - 3 Month 22.5 mg, and Lupron Depot® 7.5 mg are indicated for the palliative treatment of advanced prostate cancer. These dosages are not approved for use in women. The most common side effect associated with Lupron Depot is hot flashes. Like other treatment options, LH-RH agonists may cause impotence. Symptoms may worsen over the first few weeks of treatment. Periodic monitoring of PSA and serum testosterone levels is recommended. For further information, please see complete prescribing information below.

Lupron Depot® - 4 Month 30 mg
(leuprolide acetate for depot suspension)

Lupron Depot® - 3 Month 22.5 mg
(leuprolide acetate for depot suspension)

Lupron Depot® 7.5 mg
(leuprolide acetate for depot suspension)

**Gynecology**
Lupron Depot® - 3 Month 11.25 mg and Lupron Depot® 3.75 mg are indicated for the management of endometriosis and in combination with iron for the preoperative treatment of anemia caused by uterine fibroids. Side effects associated with Lupron Depot are generally those related to hypoestrogenism, including vasomotor flushes, headaches, and vaginal dryness. After 6 months of therapy with Lupron Depot 3.75 mg, vertebral bone density decreased by an average of 3.2%, compared with pretreatment value. For further information, please see complete prescribing

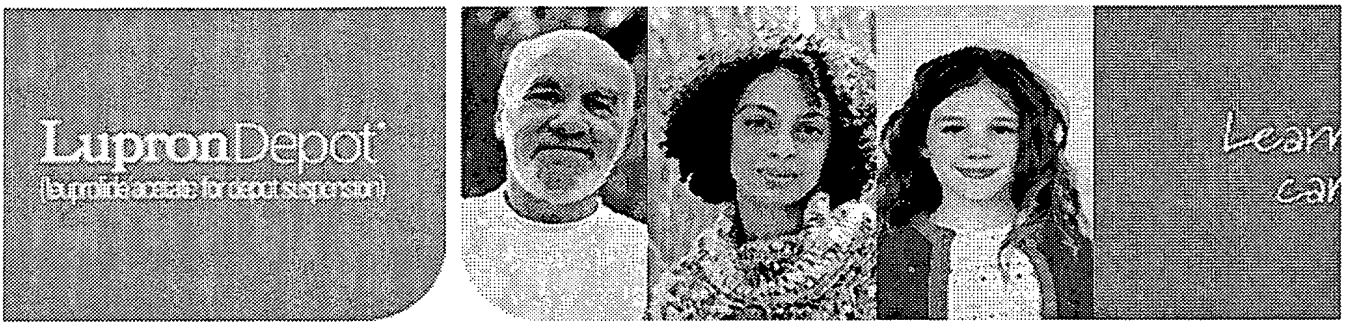
information below.

Lupron Depot® - 3 Month 11.25 mg
(leuprolide acetate for depot suspension)

Lupron Depot® 3.75 mg
(leuprolide acetate for depot suspension)

**Pediatric**
Lupron Depot-PED® 7.5 mg, 11.25 mg, and 15 mg are indicated for the treatment of children with central precocious puberty. In clinical studies, the most frequently reported adverse event related to therapy with Lupron Depot-PED was an injection site reaction seen in 5% of children in combined studies. The recommended starting dose of Lupron-PED is 0.3 mg/kg/4 weeks (minimum 7.5 mg). Inadequate dosing may result in poor control of the pubertal process. For further information, please see complete prescribing information below.

Lupron Depot-PED®
(leuprolide acetate for depot suspension)
7.5 mg, 11.25 mg and 15 mg

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## Lupron Depot®... Learn How It Can Help

If you or a loved one has been prescribed Lupron Depot®, we can help you better understand the treatment and how it works.

Lupron Depot belongs to a class of drugs called gonadotropin-releasing hormone (GnRH) agonists. It is used to decrease the body痴 production of specific hormones, natural chemicals that influence the behavior of certain cells. Because Lupron Depot can reduce the production of both male and female hormones, it is used to treat specific conditions in men, women, and children.

This site provides information on several conditions including fibroids, prostate cancer, endometriosis, and central precocious puberty.

To find out more about Lupron Depot therapy, click on the specific condition that interests you.

Please see below for important information on Lupron Depot, including side effects.

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PROSTATE CANCER: Lupron Depot (-4 Month 30 mg, -3 Month 22.5 mg, and 7.5 mg) is indicated for the palliative treatm cancer. The most common side effect associated with Lupron Depot® is hot flashes. Like other treatment options, GnRH a impotence. Symptoms may worsen over the first few weeks of treatment. Periodic monitoring of PSA and serum testoster The -4 Month 30 mg, -3 Month 22.5 mg, and 7.5 mg dosage forms are not indicated for use in women. For further inform complete prescribing information.

ENDOMETRIOSIS: Lupron Depot (3.75 mg and -3 Month 11.25 mg) is indicated in the treatment of endometriosis. Side ef related to hypoestrogenism, including vasomotor flushes, headaches, and vaginal dryness. After six months of therapy wit vertebral bone density decreased by an average of 3.2%, compared with the pretreatment value. For further information, prescribing information.

FIBROIDS: Lupron Depot (3.75 mg and -3 Month 11.25 mg), in combination with iron, is indicated for the preoperative tre with uterine fibroids. Most common side effects with Lupron Depot® are generally those related to hypoestrogenism, inclu headaches, and vaginal dryness. A small amount of bone loss (average 2.7% at month 3) may also occur during therapy. please see the complete prescribing information.

CENTRAL PRECOCIOUS PUBERTY: Lupron Depot-PED® (7.5 mg, 11.25 mg, and 15 mg) is indicated in the treatment of ce

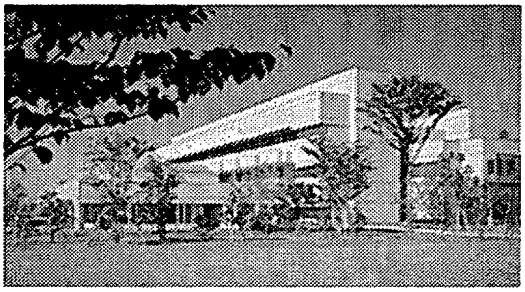
clinical studies, the most frequent adverse event related to therapy with Lupron Depot-PED was an injection site reaction, combined studies. The recommended starting dose of Lupron Depot-PED is 0.3 mg/kg/4 weeks (minimum 7.5 mg). Inade poor control of the pubertal process. For further information, please see the complete prescribing information.

## V. Takeda Medicines are Now Used Worldwide

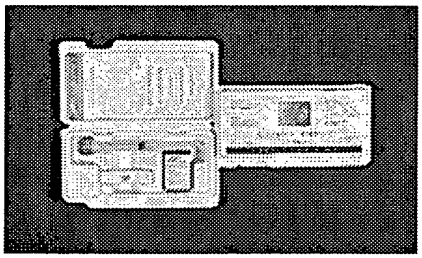
- **1985 TAP Pharmaceuticals Inc. established, begins sales of *Lupron*®**
  In 1985, Takeda formed TAP Pharmaceuticals Inc. (now TAP Pharmaceutical Products Inc.) in the U.S. in a 50:50 joint venture with Abbott Laboratories. TAP began marketing the prostate cancer treatment *Lupron*® in the same year, followed by *Lupron Depot*® (a once-monthly dosage of *Lupron*®) in 1989, and *Prevacid*® (anti-peptic ulcer agent) in 1995. TAP now has net sales surpassing $3.5 billion and is one of the world's fastest-growing pharmaceutical companies.

  Takeda currently has three operating bases in the U.S., including a holding company and development and marketing companies.

- **1988 Tsukuba Research Laboratories established**
  Tsukuba Research Laboratories became Takeda's second research base, coupled with the one in Osaka. These laboratories conduct basic research using cutting-edge technologies.



- **1989 *Lupron Depot*® launched**
  Effective for one full month with a single dosing, this was the successor to *Lupron*®. It is now sold in more than 60 countries under such brand names as *Lupron Depot*®, *Enantone*® and *Leuplin*®, and has become one of Takeda's principal products. Also on the market now are 3 Month Depot (one dosing every 3 months), and 4 Month Depot (one dosing every 4 months).



- **1991 Lansoprazole (proton pump inhibitor and anti-peptic ulcer agent) launched**
  Lansoprazole is now sold in more than 90 countries under the brand names *Prevacid*®, *Ogast*®, *Takepron*® and others. It has become Takeda's top-selling product.

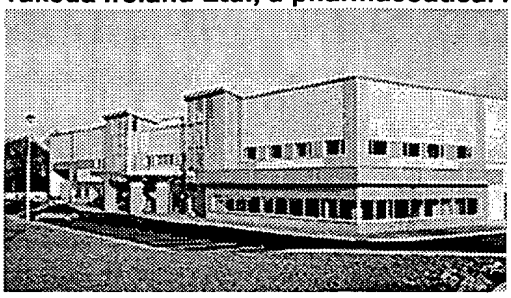
  The success of *Lupron Depot*® and *Prevacid*® helped Takeda solidify its position as a global pharmaceutical manufacturer. These two drugs each now have worldwide sales exceeding $1 billion and have given us a firm business foundation in the U.S. and Europe.

In recent years, we further solidified our overseas bases to prepare for our new blockbuster drugs and aimed at greater business expansion.

- **1997**
  **Takeda UK Limited, a wholly owned pharmaceutical marketing subsidiary, established in the U.K.**

***Blopress*® (angiotensin II receptor antagonist and anti-hypertensive agent) launched**

**Takeda Ireland Ltd., a pharmaceutical manufacturing plant, established**



Takeda Ireland Ltd.

**Takeda America Holdings, Inc., a holding company for our U.S. pharmaceutical business, established**

- **1998**
  **Majority share acquired in Takeda Italia Farmaceutici S.p.A., a pharmaceutical marketing company in Italy**

  **Laboratoires Takeda, a pharmaceutical marketing joint venture in France, becomes a wholly owned subsidiary of Takeda**

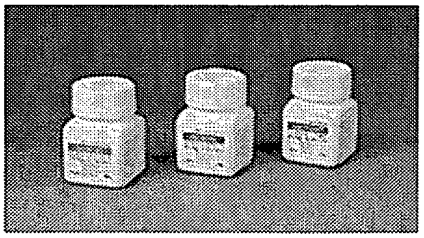
**Takeda Pharmaceuticals America, Inc. (now Takeda Pharmaceuticals North America, Inc.), a wholly owned pharmaceutical marketing company, established in the U.S.**

**Takeda Pharma AG, a pharmaceutical marketing company, established in Switzerland**

**Takeda Europe Research & Development Centre Ltd., a pharmaceutical development company, established in the U.K.**

- **1999**
  **Insulin sensitizer *Actos*® launched; U.S. sales begin in a co-promotion agreement between Takeda Pharmaceuticals America, Inc. and Eli Lilly and Company**

  **Takeda launched the anti-hypertensive agent *Blopress*® and the anti-diabetic agent *Actos*® in Japan.**



The addition of *Blopress*® and *Actos*® to *Lupron Depot*® and *Prevacid*® positions Takeda to further develop its business and increase its global market presence in the twenty-first century.

- **2000**
  **The right to access and utilize the databases of Celera Genomics obtained**

  **Animal health business transferred to a joint venture Schering-Plough Animal Health K.K.**

  ***Actos*® launched in Europe**

- **2001**
  **Takeda Pharmaceuticals America, Inc. was renamed to Takeda Pharmaceuticals North America, Inc.**

  **All the shares of Takeda Vitamin & Food USA, Inc., Takeda Canada Vitamin and Food Inc., Takeda Europe GmbH, and Takeda Vitamin & Food Asia Pte. Ltd. transferred to BASF AG**

  **Urethane chemicals business transferred to a joint venture Mitui Takeda Chemicals Inc.**

  **The erectile dysfunction treatment *Ixense*® launched in Europe**

  **Takeda Research Investment,Inc.,investment company,established in U.S.**

- **2002**
  **All the Shares of Takeda Pharma GmbH aquired, a pharmaceutical marketing company in Germany**

  **Food business transferred to a joint venture Takeda Kirin Foods Corporation**

  **Benet® launched in Japan**

PREV